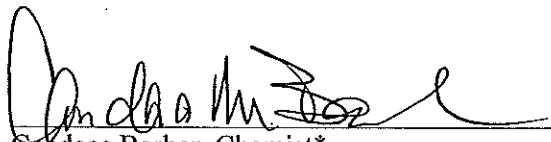


**Standard Operating Procedure (SOP) for**

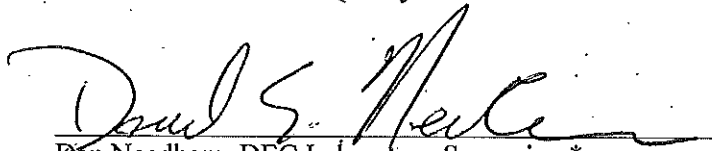
**Determination of Phosphorus by Flow Injection Analysis  
(Acid Persulfate Digestion Method)**

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\*Signature implies that the individual has read, understands and agrees to follow this Standard Operating Procedure.

\*\*Signature indicates SOP has been revised.

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## **1.0 Identification of Test Method**

- 1.1 This SOP is for the analysis of Phosphorus either total or dissolved and is based on Standard Methods for the Examination of Water and Wastewater, 21<sup>th</sup> Ed. APHA-AWNA/WPCF Method 4500-P H Manual Digestion and Flow Injection Analysis for Total Phosphorus and Determination of Total Phosphorus by Flow Injection Analysis Colorimetry, QuikChem Method 10-115-01-1-F.
- 1.2 Orthophosphate (SRP) procedure is exactly the same as TP minus digestion. Holding time is 48 hours.

## **2.0 Applicable Matrix/Matrices**

- 2.1 This method determines the amount of phosphorus in drinking, ground, surface water and soil extracts.

## **3.0 Method Detection Limit (MDL)/Limit of Quantitation (LOQ)**

- 3.1 The MDL for both Total Phosphorus and Dissolved Phosphorus is 2.5µg P/L.
- 3.2 The LOQ for both Total Phosphorus and Dissolved Phosphorus is 5µg P/L.

## **4.0 Scope and Application**

- 4.1 This method covers the determination of total phosphorus in drinking water, non-potable water and laboratory DI water. This method determines total phosphorus in non-filtered samples and total dissolved phosphorus in samples that were filtered through a 0.45µm filter. The difference between the result of a sample determined directly and filtered is termed total insoluble phosphorus.
- 4.2 The method is based on reactions that are specific for the orthophosphate ( $\text{PO}_4^{3-}$ ) ion.
- 4.3 The applicable range of the 5 - 200µg P/L.

## **5.0 Summary of Test Method**

- 5.1 The orthophosphate ion  $\text{PO}_4^{3-}$  reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex, which absorbs light at 880nm. The absorbance is proportional to the concentration of orthophosphate in the sample. Polyphosphates may be converted to the orthophosphate form by sulfuric acid digestion and organic phosphorus may be converted to orthophosphate by persulfate digestion.

## **6.0 Definitions**

- 6.1 Calibration Blank (CB) - A volume of reagent water in the same matrix as the calibration standards, but without the analyte.
- 6.2 Calibration Standard (CAL) - A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
  - 6.2.1 Continuing Calibration Verifications (CCV and CCB) - Calibration standards analyzed at regular intervals used to monitor the instrument calibration. Refer to section 6.14 for DQM

- 6.2.2 CCV Mid – The CCV Mid is a mid-level calibration standard that is analyzed at the beginning of analysis, after every 10 samples and at the end of analysis.
- 6.2.3 CCV Low – The CCV Low is low-level standard that is two times the PQL (6.11). It is analyzed at the beginning of the run.
- 6.2.4 CCB – A blank calibration standard used to monitor the instrument calibration during analysis. It is analyzed at the beginning of analysis, after every 10 samples and at the end of analysis.
- 6.3 Method Blank (MB) – A laboratory reagent water blank that is treated exactly as a sample, and is used to monitor lab contamination.
- 6.4 Laboratory Control Sample (LCS) – A laboratory reagent blank spiked with known amounts of analyte. The LCS is used to assess the performance of all or a portion of the measurement system. The LCS samples are then analyzed and processed exactly as customer samples.
  - 6.4.1 LCS – Mid – The target analyte concentration is at mid-calibration level.
  - 6.4.2 LCS – Low – The target analyte concentration is at or near the PQL.
- 6.5 Filter Blank (FB) - A laboratory reagent water blank that is treated exactly as a sample, and is used to monitor lab contamination. A filter blank is analyzed only when sample filtration is required at the bench.
- 6.6 Field Split (FS) Sample – An aliquot of a well-mixed sample is poured into two separate containers by field personnel under field conditions. These Duplicates are processed and analyzed independently either as Duplicates (6.7.1) or Matrix Spikes (6.7.2). One of the FS Samples is logged into the lab data system and its corresponding Lab ID# is written on the label of the second FS Sample. The goal for the fiscal year is for the lab to receive 10% of the total sample TP/DP load as FS Samples.
  - 6.6.1 Duplicate – A routine environmental (FS) sample analyzed to obtain a measure of precision.
  - 6.6.2 Matrix Spike (MS) – A sample prepared by adding a known volume of target analyte to a specified amount of matrix sample for which an independent estimate of target analyte concentration is available. Matrix spikes are used to determine recovery efficiency. The added analyte should have the same concentration as the LCS – Mid (6.5.1).
- 6.7 Initial Calibration Verification (ICV) – The ICV is obtained from a source other than the calibration standards. The ICV is a sample to assess the performance of all or a portion of the measurement system. The analyte concentration of the ICV is typically at mid-calibration level. The ICV sample is analyzed and processed exactly as customer samples.
- 6.8 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 6.9 Limit of Detection (LOD) – The LOD is the lowest concentration level that can be statistically different from a blank. The LOD is approximately equal to MDL.
- 6.10 Limit of Quantitation – The LOQ is approximately equal to the PQL. A CCV-Low (6.3.2) is included in each analysis at 1-2 times the PQL.

- 6.11 Practical Quantitation Limit (PQL) – The laboratory reporting limit, which is 2 to 5 times the MDL (6.8).
- 6.12 Proficiency Evaluation (PE) – A certified solution of method analyte to test whether the analyst/laboratory can produce analytical results within specified acceptance criteria.
- 6.13 Digestion Prep Batch – The set of 20 samples, a MB and an LCS all digested at the same time.
- 6.14 Analytical Batch – Digested Phosphorus samples all analyzed at the same time. An analytical batch can include many digestion prep batches (6.14).
- 6.15 Data Quality Management (DQM) – The DQM is a Lachat Omnion Software specific term used within a Run Worksheet/Tray by defining properties of the samples DQM Tests, DQM Pass and Fail Messages, and DQM Pass and Fail Actions. DQM sets are defined within the Run Worksheet/Tray. After all customer samples have been entered into the Run Worksheet/Tray, the CCV/CCB (6.3) is defined as the DQM set which is then scheduled to run after 10 samples and at the end of the run. This ensures that all samples are bracketed by the method required CCBs and CCVs. This repeating DQM Set will start at the current row in the Run Worksheet/Tray, so it is important this first occurrence of the DQM Set is in the correct row before setting its schedule.

## 7.0 Interferences

- 7.1 Silica forms a pale blue complex, which also absorbs at 880nm. This interference is generally insignificant, as a silicate concentration of approximately 30mg SiO<sub>2</sub>/L would be required to produce a 0.005mg P/L positive error in orthophosphate.
- 7.2 Concentrations of ferric iron greater than 50mg Fe<sup>+3</sup>/l will cause a negative error due to competition with the complex for the reducing agent ascorbic acid and the subsequent loss of orthophosphate. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.
- 7.3 Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate causing a negative error.
- 7.4 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 10% HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

## 8.0 Safety

- 8.1 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 as low as reasonably achievable.
- 8.2 Material Safety Data Sheets (MSDS) are available to all personnel involved in the chemical analysis. The following chemicals have the potential to be highly toxic:
- 8.3 Personal Protective Equipment (PPE) should be used where appropriate.

- 8.4 Maintain working areas in a safe manner, which includes cleaning of bench tops, putting away chemicals and glassware after use, cleaning spills and keeping general clutter to a minimum.

## 9.0 Equipment and Supplies

- 9.1 Dedicated Class A glassware that has been acid soaked in 10% HCL and rinsed several times with D.I. H<sub>2</sub>O.
- 9.2 60mL pre-cleaned disposable vessels with PTFE lined screw caps (QEC Item # 2112-60mlC).
- 9.3 Autoclavable Test Tube Racks.
- 9.4 Balance - Analytical, capable of accurately weighing to the nearest 0.0001g.
- 9.5 Lachat QuickChem 8000 Flow Injection Analysis instrument.
- 9.6 Lachat Omnion 3.0 Software.
- 9.7 Automatic Pipettes ranging from 100µl to 10ml.
- 9.8 Autoclave.

## 10.0 Reagents and Standards

- 10.1 Reagent preparation must be recorded in the Nutrients Reagent Logbook located next to the analytical balance and must include the following:
- 10.1.1 Preparation date and chemist's initials (i.e. mmddyyDGM).
- 10.1.2 Manufacturer with lot number of chemical or prep date (i.e. mmddyyDGM) of chemical being used.
- 10.2 The prep dates of all reagents used for each sample analysis must be recorded on the TP/DP Standards/QC Spreadsheet found on the Phosphorus computer.
- 10.3 The preparation dates of all Calibration Standards, Spike Solutions and Quality Control Check Samples are recorded on the TP/DP Standards/QC Spreadsheet. A copy of this spreadsheet is printed and included with the final data package.
- 10.4 Reagents
- 10.4.1 Ammonium Persulfate – Sigma Aldrich Cat # 248614-500g
- 10.4.2 Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) – Fisher Cat # A300SI-212
- 10.4.3 Ammonium Molybdate Tetrahydrate – Sigma Aldrich Cat # A7302-500g
- 10.4.4 Antimony Potassium Tartrate Trihydrate – Fisher Cat # A867-250
- 10.4.5 Dodecyl Sulfate Sodium Salt, 99% (SDS) – Acros Cat # 23042-1000
- 10.4.6 Ascorbic Acid – Fisher Cat # A62-500
- 10.5 Digestion Reagents
- 10.5.1 Ammonium Persulfate Digestion Reagent – Prepared daily  
40g Ammonium Persulfate (10.4.1) → 100mL in DI H<sub>2</sub>O.  
Based on the above recipe, prepare enough of the reagent by adding reagent to a graduated cylinder and diluting to volume with DI H<sub>2</sub>O.  
Cover the cylinder with Parafilm and invert to mix. Reagent volume is 1mL per test tube.
- 10.5.2 11N H<sub>2</sub>SO<sub>4</sub> – Caution: Strong exothermic Reaction...Always add acid to water  
In a 1L volumetric flask, slowly and carefully add 310mL Concentrated

H<sub>2</sub>SO<sub>4</sub> (10.4.2) to 600mL DI H<sub>2</sub>O. Allow to cool. Dilute to 1L mark with DI H<sub>2</sub>O. Expiration date is 1 year from preparation date. Reagent volume is 1mL per 50mL sample.

## **10.6 Analytical Reagents**

### **10.6.1 Carrier and Diluent Reagent (Acid Wash) – Expiry date of 1 year.**

Fill a 2L volumetric flask with approximate 1.5 liters of DI H<sub>2</sub>O. Add 40mL 11N H<sub>2</sub>SO<sub>4</sub> (10.4.2) and 16g Ammonium Persulfate (10.4.1). Dilute to 2L mark with DI H<sub>2</sub>O. Pour into an autoclavable polycarbonate container. Digest in the autoclave the Acid Wash for 30 minutes at 121°C and 15psi exposure. Allow to cool before use.

### **10.6.2 Ascorbic Acid Reagent – Expiry date of 5 days**

Fill a 1L volumetric flask with approximately 700mL of DI H<sub>2</sub>O. Add 60.0g Ascorbic Acid (10.4.6). Dilute to 1L mark with DI H<sub>2</sub>O and invert to mix. Pour into a 1L plastic container and add a stir bar. Add 1.0g of SDS (10.4.5). Place on stir plate and allow to mix for a few minutes. Discard solution if the solution becomes yellow.

### **10.6.3 Stock Ammonium Molybdate Reagent – Expiry date of 30 days**

In a 500mL volumetric flask dissolve 20.0g Ammonium Molybdate Tetrahydrate (10.4.3) in approximately 300mL DI H<sub>2</sub>O. Dilute to mark with DI H<sub>2</sub>O, cover with parafilm and stir for a minimum of four hours (can be left stirring overnight). Store in plastic and refrigerate.

### **10.6.4 Stock Antimony Potassium Tartrate Reagent – Expiry date of 30 days**

In a 100mL volumetric flask dissolve 0.3g Antimony Potassium Tartrate (10.4.4) in approximately 80mL DI H<sub>2</sub>O. Dilute to mark with DI H<sub>2</sub>O, cover with parafilm and invert to mix. Store in a dark bottle and refrigerate.

### **10.6.5 Molybdate Color Reagent – Expiry date of 5 days**

Fill a 1L volumetric flask with approximately 500mL DI H<sub>2</sub>O. Add 21.0mL H<sub>2</sub>SO<sub>4</sub> (10.4.2). Swirl to mix. Add 72.0mL Stock Antimony Potassium Tartrate Reagent (10.4.4). Swirl to mix. Add 213mL Stock Ammonium Molybdate Reagent (10.6.3). Swirl to mix. Dilute to mark with DI H<sub>2</sub>O, cover with parafilm and invert to mix.

## **10.7 Standards and Spike Solutions**

### **10.7.1 All calibration standards and ICVs must be digested as in section 14.0 in the same manner as unknown customer samples.**

### **10.7.2 1000mg/L Phosphate-Phosphorus – Spex CertiPrep Cat # AS-PO4P9-2Y**

### **10.7.3 100µg P/L ICV Standard – Expiry date of 30 days**

Measure 50mL DI H<sub>2</sub>O in a pre-cleaned 60mL test tube with screw cap (9.2). Add 500µL of 1000mg/L Phosphate (10.6.2). Refer to section 14.0 for digestion.

### **10.7.4 Intermediate (Int) Calibration (Spex) Stock Standards – Expiry date of 28 days**

- a) **10mg P/L Int Cal Stock**  
1mL (10.7.2) → 100mL in DI H<sub>2</sub>O
- b) **1mg P/L Int Cal Stock**  
100µL (10.7.2) → 100mL in DI H<sub>2</sub>O

**10.7.5 Working Calibration Standard Prep – Expiry date of 30 days**

- a) Prepare the calibration by using the following volumes of 10mg/L (10.7.4a) or 1mg/L (10.7.4b). Standards are prepared in DI H<sub>2</sub>O.

**Table 1**

Concentration (µg P/L)	Recipe
200µg P/L	4mL of 10mg/L (10.7.3a) → 200mL
100µg P/L	2.5mL of 10mg/L (10.7.3a) → 250mL
50µg P/L	1mL of 10mg/L (10.7.3a) → 200mL
10µg P/L	2mL of 1mg/L (10.7.3b) → 200mL
5µg P/L	1mL of 10mg/L (10.7.3b) → 200mL
0µg P/L	200mL DI H <sub>2</sub> O

- b) Using a graduated cylinder, measure 50mL aliquots of each standard and pour into pre-cleaned 60mL screw top test tubes (9.2). Refer to section 14.0 for digestion.

**10.7.6 1000mg/L Phosphate as P – ERA Cat # 061**

**10.7.7 Intermediate (ERA) LCS/MS Stock Standards – Expiry date of 28 days**

- a) **10mg P/L Int LCS/MS Stock**  
1mL (10.7.6) → 100mL in DI H<sub>2</sub>O
- b) **1mg P/L Int LCS/MS Stock**  
100µL (10.7.6) → 100mL in DI H<sub>2</sub>O

**10.7.8 LCS, LCS Low and MS Prep**

- a) Measure 50mL of DI H<sub>2</sub>O in pre-cleaned 60mL test tubes with screw caps (9.2) for the LCS and the LCS Low. Pre-measured samples are provided to the lab by field personnel to use for MS Prep.

**Table 2**

	Volume of ERA Stock	Volume Matrix	Concentration
LCS Low	250µL 1mg/L (10.7.7b)	50mL DI H <sub>2</sub> O	100µg P/L
LCS	500µL 10mg/L (10.7.7a)	50mL DI H <sub>2</sub> O	100µg P/L
MS	500µL 10mg/L (10.7.7a)	50mL Sample	100µg P/L

**11.0 Sample Collection, Preservation, Shipment and Storage**

- 11.1 Pre-cleaned 60mL test tubes with PTFE lined screw caps (9.2) are to be marked to show the 50mL fill-to line using a sharpie and 'jig' prior to sending the test tube out to samplers by lab personnel. All TP and DP samples are collected in these pre-marked/pre-cleaned 60mL test tubes.
- 11.2 Samples being analyzed for DP are filtered through a 0.45µm membrane filter by field personnel. Field personnel are to provide and login a filter blank sample for lab analysis.
- 11.3 Field personnel will provide field split samples which will be used for required



laboratory quality control purposes as duplicates and matrix spikes.

11.4 Samples are not preserved prior to digestion. Samples have a hold time of 28 days once digested.

11.5 Samples are received via drop off by field personnel or the mail. 10.8.6 Samples are stored at room temperature. Undigested samples (samples as received into the lab) do not need to be stored in secondary containment. Digested samples, which contain oxidizers and have a pH < 2, must be stored in some form of secondary containment.

## 12.0 Quality Control

12.1 The laboratory operates a formal Quality Control (QC) program. The minimum requirements of this program consist of an initial demonstration of capability, the analysis of method blanks, LCS's, sample matrix spikes, duplicates, and ICV as checks on the analytical performance. The laboratory maintains records that monitor the quality of data.

12.2 Initial Demonstration of Capability (IDC) – The date and analyst's initials of the following are to be documented on the Demonstration of Capability Certification Statement sheet and filed appropriately in the trainee's training folder:

12.1.1 The review of relevant reference method(s)

12.1.2 The review of the laboratory SOP

12.1.3 Observation of analysis by current analyst

12.1.4 Analysis of samples with little to no supervision

12.1.5 Analysis of four LCS or ICV samples.

12.1.6 The completion date of training

12.1.7 Signatures of the trainee, trainer and the quality assurance officer.

## 12.3 Calibration

12.3.1 The Initial Calibration must use a minimum of five standards and a blank. All standards are run in replicates of 2 as an Indication of Instrument Precision. The lowest calibration standard should be at the reporting limit and the highest concentration at the upper end of the calibration range ensuring that the calibration range encompasses the expected concentration values of the samples or required dilutions.

a) Indication of Instrument Precision – comparison between replicates of each calibration standard. The acceptance criteria is  $\leq 10\%$  RSD between each standard.

12.3.2 Analyses of the CCB and the CCV Mid demonstrate the ongoing precision and recovery and are analyzed on an ongoing basis. They are analyzed at the beginning of the run, again every ten samples and at the end of the run.

a) CCB acceptance criteria is  $\leq \frac{1}{2}$  of the PQL.

b) CCV Mid acceptance criteria is  $\pm 10\%$  recovery of the target value.

12.3.3 Analysis of an ICV verifies the calibration curve through a secondary source. It is analyzed at the beginning of the run. ICV acceptance criteria is  $\pm 10\%$  recovery of the target value

12.3.4 Analysis of a CCV Low demonstrates the ongoing precision and recovery at the low end of the calibration curve. It is analyzed at the beginning of

the run. Acceptance criteria for the CCV Low  $\pm 10\%$  recovery of the target value.

#### **12.4 Laboratory Performance**

**12.4.1** Analyses of a Method Blanks are required to demonstrate freedom from contamination. One Method Blank is required per Digestion Prep Batch of 20 samples. Acceptance criteria for the Method Blank  $\leq \frac{1}{2}$  of the PQL.

**12.4.2** Analyses of LCS Mids are required to evaluate laboratory performance and analyte recovery in a blank matrix. One LCS Mid is required per Digestion Prep Batch of 20 samples. Acceptance criteria for the LCS Mid  $\pm 10\%$  recovery of the target value.

**12.4.3** Analyses of Matrix Spikes (MS) and Duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences caused by the sample. Field Split Samples are provided to the laboratory by field staff for the use of matrix spikes or duplicates. The yearly goal is for 10% of the total yearly samples to have been analyzed as duplicates and as matrix spikes.

a) MS acceptance criteria  $\pm 15\%$  recovery of the target value.

b) Duplicate acceptance criteria  $\leq 10\%$  RPD for samples  $\geq$  the PQL.

**12.4.4** Analysis of LCS Low is required to evaluate the laboratory performance of the method and analyte recovery at the reporting limit. One LCS Low is required per daily TP digestion and daily DP digestion. Acceptance criteria  $\pm 30\%$  recovery of the target value.

**12.4.5** Proficiency Evaluation – A sample with an unknown amount of analyte is analyzed to test whether the analyst/laboratory can produce analytical results within specified acceptance criteria. It is analyzed annually.

#### **13.0 Calibration and Standardization**

**13.1** Prepare reagents and standards as described in section 10.0.

**13.2** Set up manifold as shown in Appendix 1.

**13.3** Input data system parameters as shown in Appendix 1.

**13.4** Pump DI H<sub>2</sub>O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

**13.5** Place standards in the sampler. Input the information required by the data system.

**13.6** Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve. Acceptance criteria is  $\geq 0.995$ .

**13.7** Verify calibration using a CCV Low, CCV Mid and CCB as described in section

**13.8** If percent recovery exceeds acceptance criteria as described in section 18.0, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

## **14.0 Procedure**

### **14.1 Digestion Procedure**

**Note:** Samples may be diluted prior to digest when high concentrations can be predicted.

**14.1.1** 60mL test tubes with a 'Collect sample for Duplicate or Spike' label are provided to the lab by field personnel for required laboratory duplicate or matrix spike quality control purposes. The analyst will decide at the time of analysis whether to use the sample as a Duplicate or as an MS by clearly marking the label. The samples designated as matrix spikes will be set aside for fortifying with spike solution as per section 10.6.8.

**14.1.2** Sample container should contain 50mL as indicated by the black mark on the 60mL test tube. Shake well and decant excess sample if required.

#### **14.1.3 TP Sample Digestion**

- a) Prepare 1 LCS Low (10.7.8) per daily digestion prep batch.
- b) Prepare 1 Method Blank and 1 LCS Mid (10.7.8) per digestion prep batch of 20 samples.

#### **14.1.4 DP Sample Digestion**

- a) Prepare 1 LCS Low (10.7.8) per daily sample digestion batch.
- b) Prepare 1 Method Blank and 1 LCS Mid (10.7.8) per sample digestion batch of 20 samples.

#### **14.1.5 TP/DP Calibration Standards and ICV**

- a) Prepare calibration standards as per 10.7.

**14.1.6** Calibration standards, QC and samples are digested using the following procedure:

- a) Set up samples in autoclaveable racks.
- b) Uncap all samples.
- c) Dispense 1mL Ammonium Persulfate Digestion Reagent to each sample.
- d) Dispense 1mL 11N H<sub>2</sub>SO<sub>4</sub> (10.5.2) to each sample.
- e) Replace caps securely.
- f) Label racks with Date of Digestion. Autoclave samples for 30 minutes at 15 psi and 121°C (use the pre-programmed autoclave method P6). Record the sample type (TP, DP, Standards or Acid Wash), digestion date, initials and autoclave program being used.
- g) After cycle is complete, remove racks from autoclave and allow to cool before analysis.

### **14.2 Creating a Run Worksheet and Sample Tray Setup Procedure**

**14.2.1** Open Omnion 3.0 Icon.

**14.2.2** Click on: Run → Open → ....Omnion → Data folder.

**14.2.3** Select Phosphorus Total for the TP worksheet template or select Dissolved Phosphorus for the DP worksheet template. Three windows will appear on the screen: the Run Worksheet, the Run Properties and one Channel Data Display.

#### **14.2.4 Calibration Standard Tray Setup**

- a) The Cup No.'s (1<sup>st</sup> column of the Run Worksheet) used for the standards are identified as S1, S2, S3, etc.

- b) The Calibration Standard/Sample Type (3<sup>rd</sup> column of the Run Worksheet) rows will be highlighted a blue color.
- c) The CCB, CCV Mid, CCV Low and ICV Mid Check Standards rows will be highlighted a green color.
- d) Uncap and place 60mL test tubes containing digested standards into standards tray that corresponds with the Cup No. on the Run Worksheet.

#### 14.2.5 Sample Tray Setup.

- a) The Cup No.'s (1<sup>st</sup> column of the Run Worksheet) are identified as 1, 2, 3, etc. in the Run Worksheet. The Cup No. refers to the labeled position number in the sample rack.
- b) Working with one digested sample at a time, begin scanning or manually entering the samples in the Sample ID column (the 2<sup>nd</sup> column in the Run Worksheet screen). Once entered, pour an aliquot of sample into a 7mL test tube and place into the tray position that corresponds with the Cup No. (1<sup>st</sup> column of the Run Worksheet).
  - 1. To delete extra rows, click and drag along the selected rows, then right-click and select Delete.
  - 2. To add new rows to the end of the spreadsheet, select any row, then right-click to get the edit menu. Click on the Append Row, to add one row, or on Append Many, to add more than one row.
  - 3. To insert a row or rows before or above a row, click on the row to select it, right-click, then click on Insert Row or Insert Many.
  - 4. To Auto Number Cups, click and drag on the rows in the spreadsheet in which the Cup No. sequence should appear. The right-click and click on Columns, Auto Number Cups. All Samples rack Cup Nos., those not in the Standards rack (S1, S2, etc.) will be renumbered sequentially, incrementing by 1, starting with the first non-Standards rack Cup No. of the selection.
- c) Place the sample tray in the auto-sampler. Make sure the tray is positioned properly in the auto-sampler tray. Sample trays are placed from left to right in the auto-sampler. Three racks of 90 samples can be placed in the auto-sampler.
- d) Place a rack of empty 7mL test tubes last space on the right of the auto-sampler. These are to be used for dilutions by auto-diluter.

#### 14.2.6 Defining DQM

Click and drag the CCB and CCV Mid green Check Standard rows. Right-click and select Define DQM Set. Select "After every N samples". Enter #: 10 in the empty field. Make sure the Close End of Run box is checked. Click OK. The DQM Set is now scheduled to appear at the beginning of the run, every 10 samples and at the end of the run.

### 14.3 Calibration and Sample Analysis

#### 14.3.1 Setup manifold and manifold pump tubing.

#### 14.3.2 Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate.

#### 14.3.3 From the main menu, select Configuration → Autosamplers to open the

Configure Autosamplers window.

- a) Click on Initialize Autosampler to initialize communication between the autosampler and the computer.
- b) Click on Prime Dilutor to prepare the autodilutor for samples needing dilution.
- c) Click Close to return to the Run Worksheet window.

**14.3.4** From the main menu, select the Start icon to begin instrument calibration and sample analysis. The data system will then associate the concentrations with the instrument responses for each standard.

#### **14.4 End of Sample Analysis**

**14.4.1** Remove the TP Color Reagent manifold line from the reagent. Place the line a 0.1N – 0.3N NaOH solution and allow 1-3 inches of the solution to pump through the line. Remove the line out of the NaOH solution and place into a flask containing DI H<sub>2</sub>O and allow to pump for a few minutes.

**14.4.2** Remove all other manifold lines from their reagents and place into the flask containing DI H<sub>2</sub>O and allow to pump for a few minutes.

**14.4.3** Remove all manifold lines from the DI H<sub>2</sub>O and allow air to pump through the manifold lines for a few minutes.

**14.4.4** Turn off the manifold pump and unclamp the manifold pump lines.

**14.4.5** Re-cap calibration standards, reagents and waste container. Refer to section 21.0 for sample waste disposal.

#### **14.5 Data Acquisition and Reduction**

##### **14.5.1 Export Data to File**

- a) Once the analysis is complete, insert a thumb drive into the computer's USB port. In the Runs Properties window (upper right of the screen), click on the Run tab. Click on *Export Data to File*. This action will copy the data to a USB thumb drive.

##### **14.5.2 Format and Print Run Report**

- a) Use the cursor to highlight the Calibration peaks in the Channel Data Display window.
- b) In tool bar, click on Report, and Open Format. Choose TP for Phosphorus-Digested or DP for Phosphorus Filtered/Digested format.
- c) Click on the Custom Report Format icon (which is the 8<sup>th</sup> icon (yellow) from the left in the toolbar) to display the Custom Report Format window. Click on the Layout Tab to adjust the look of the report which includes the analyst's name and analysis date. Click Apply once all changes have been made and then click Close. Refer to the Omnion 3.0 Software User Guide if other adjustments to the report need to be made.
- d) Print analytical run report. Click on Print icon, 7<sup>th</sup> from the left. Select *Print*.
- e) Save report format. Go to tool bar, click on Report, and Save Format. Choose TP for Phosphorus-Digested or DP for Phosphorus Filtered/Digested and click OK. Close window

**14.5.3** Print a copy of the TP/DP Standards/QC spreadsheet to include with the

analytical run report.

- 14.5.4 Save an electronic copy of the TP/DP Standards/QC spreadsheet by clicking on the 'Save' radio button located on the spreadsheet.
- 14.5.5 Use the cursor to highlight the Calibration peaks in the Channel Data Display window.

#### 14.6 Parse the Data

- 14.6.1 Insert the thumb drive into USB port on a DEC Sample Master LIM System networked computer.
- 14.6.2 Open Windows Explorer. Open the following: Y Drive > LIMS > Instrument Parsers Folder > Lachat Omnion 3 folder > Lachat Omnion 3 Parser.
- 14.6.3 Enter analyst initials, confirm analysis date and OK. At the prompt, go to the E: / drive and choose the file to be parsed. Parsing will commence. Data will be displayed in a color coded format. Review data, QC numbers, recovery calculations and formulas. Highlight and set the Print Area to be printed. Print a copy. Click on the Save icon. Minimize this screen.
- 14.6.4 Enter the QC Batch ID (refer to 12.7 for creating a QC Batch) in the window and click OK. Parsing will finish and data will be configured for import into the DEC Sample Master LIM System. Review one last time. Click the Save Icon and close the screen.

#### 14.7 Create a QC Batch

- 14.7.1 Log in to the DEC Sample Master LIM System. Click on the 'Data Entry' icon in the Main Menu window. Highlight the 'Create QC batch' then click on the 'Select' option.
- 14.7.2 In the 'Matrix' drop down box select 'water'.
- 14.7.3 In the 'Test' drop down box select 'Phosphorus-Digested', or 'Phosphorus-Filtered/Digested'. Make sure that the 'Unassigned Samples' box is checked. Select 'Retrieve'.
- 14.7.4 In the 'QC Batch' window, check the boxes of the sample numbers to be included in the QC batch.
- 14.7.5 Click the 'New' button. A QC Batch ID numbered is automatically generated by the Sample Master LIM System to all the checked samples. This QC Batch ID number is required to complete the parsing process (refer to section 14.6.4). The date of sample digestion is considered the date of analysis. If the date of digestion is different from the date of the QC Batch ID, change it to reflect the date of digestion. Write this QC batch number on the front page of the colored parsed data report.
- 14.7.6 The 'New QC batch' window will appear. Click the 'Advanced' button. The 'New QC batch - Sequence' window will appear. Move desired type and frequency of QC from left side of window into the run sequence section on the right. After this is done, click 'Close'. This will return you to the 'QC Batch' window.

14.7.7 In the 'QC Batch' window, use the drop down boxes to assign order and sample numbers for each Duplicate(s) and Matrix Spike(s). After this is done, click the 'Add Samples' button. The check marks in sample boxes will disappear. Click 'Close'.

14.7 Import Data

14.7.1 In the Sample Master LIM System, click on the red/green icon 2nd from the bottom. Highlight the phrase *Import All Files in One Directory* and click *Select*.

14.7.2 In the dropdown box choose Lachat as the directory to import data from. Click *Import Immediately*. Sample Master will display *Do you wish to delete the completed task?* Click *No*. Close import window. Click on the 2nd icon from top and select Result Entry. Review the imported data.

14.7.3 Forward the analytical run report to another analyst for review and validation. Validated data is then authorized for reporting by the lab supervisor.

15.0 Calculations

15.1 Duplicate Sample – Relative Percent Difference (RPD)

$$RPD = \left( \frac{|\text{Sample Result} - \text{Duplicate Result}|}{((\text{Sample Result} + \text{Duplicate Result})/2)} \right) 100\%$$

15.2 Matrix Spike (MS) Sample – Percent Recovery

$$\% \text{ Recovery} = \left( \frac{(\text{MS Sample Result} - \text{Sample Result})}{\text{Known MS added Concentration}} \right) 100\%$$

15.3 LCS, CCV, ICV Samples – Percent Recovery

$$\% \text{ Recovery} = \left( \frac{\text{Found Value}}{\text{True Value}} \right) 100\%$$

16.0 Method Performance (See section 18.0)

16.1 Method Detection Limit (MDL)

16.1.1 To determine MDL values, seven aliquots of reagent water fortified at approximately half the PQL are processed through the entire analytical method.

16.1.2 Perform all calculations defined in the method and report the concentration values in the appropriate units (µg P/L).

a)  $MDL = (t) \times (s)$

(t) = 3.14 Student (t) value for 7 replicates

(s) = standard deviation of the 7 replicates

16.1.3 The MDL is determined annually, when there is a new operator, or when there is a significant change in the background or instrument response.

- 16.2 Practical Quantitation Limit (PQL) – The PQL is the lowest concentration level achievable within specified limits during routine laboratory operations. It is typically 2 – 5 times the calculated MDL. The PQL for this method is 5.0 ppb.
- 16.3 Performance Evaluation (PE) Tests – Participation in periodic PE Tests, such as USGS studies, are analyzed semi-annually. If failures occur, corrective action is taken until acceptable performance is achieved.

### 17.0 Pollution Prevention

- 17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation.
- 17.2 Laboratory policy is to purchase reagents and standards on an as needed basis and log them into the chemical inventory database and assigning an expiration date, eliminating the need for a stockroom. The database is reviewed on an ongoing basis for expired reagents that can be updated and used for another year

18.0 Table 3 - Data Assessment and Acceptance Criteria for Quality Control Measures

Assessment	QC Procedure	Frequency	Limits	Corrective Action
Calibration Curve Linearity	Correlation coefficient	Each calibration	$\geq 0.995$	Re-calibrate.
Indication of Instrument Precision	Duplicate measurements of Calibration Standards	All standards	$\leq 10\%$ RSD	Re-calibrate.
Assessment of Method Performance against second source standard	ICV Mid	Beginning of analysis	$\pm 10\%$	Re-analyze. If still out of control, find and correct source of problem. Re-calibrate the instrument if the problem is instrument related. Re-prepare a new ICV Mid if the problem is sample prep related.
Calibration Curve Stability	CCB	Beginning of analysis, every 10 samples and at end of run analysis	$\leq 1/2$ PQL	Re-analyze. If still out of control, find and correct source of problem. Re-calibrate the instrument if the problem is instrument related. Re-prepare a new CCB if the problem is sample prep related.
Calibration Curve Stability	CCV Mid	Beginning of analysis, every 10 samples and at end of run analysis	$\pm 10\%$	Re-analyze. If still out of control, find and correct source of problem. Re-calibrate the instrument if the problem is instrument related. Re-analyze any and all samples that are not bracketed by passing CCV Mids. However, if a high CCV is recorded and the samples are non-detects, results may be reported with no remark codes if necessary.
Calibration Curve Stability	CCV Low	Beginning of analysis	$\pm 10\%$	Re-analyze. If still out of control, find and correct source of problem. Re-calibrate the instrument if the problem is instrument related. Note that the CCV Low is 1-2 times the PQL which is $\approx$ LOQ
Lab Contamination Monitor	Method Blank	1 per 20 samples	$\leq 1/2$ PQL	Re-analyze. If still out of control, find and correct source of problem. The PQL for samples not bracketed by two passing Method Blanks will be adjusted to reflect a potential high bias due to lab contamination.



Assessment	QC Procedure	Frequency	Limits	Corrective Action
Assessment of Method Performance	LCS	1 per 20 samples	$\pm 10\%$	Re-analyze. If still out of control, remark code and comment in sample master.
Sample Matrix Effect on Accuracy of Results	MS	Varies per digestion batch	$\pm 15\%$	Remark code and comment in sample master.
Assessment of Method Performance at the PQL level	LCS Low	1 per digestion batch	$\pm 30\%$	Re-analyze. If still out of control, remark code and comment in sample master.
Sample Matrix Effect on Precision of Results	Duplicate	Varies per digestion batch	$\pm 15\%$	Remark code and comment in sample master.

## 19.0 Corrective Actions for Out of Control Data (See section-18.0)

- 19.1 Performing routine instrument maintenance such as changing manifold pump tubing, manifold tubing, and other fittings prior to analysis may correct for baseline issues, flow issues, etc. The Lachat Analyzer used primarily for chlorides, nitrates, etc. can also be used as a backup analytical system.
- 19.2 Contact the lab supervisor, nutrients technical director, QC officer with any questions and/or concerns.
- 19.3 For technical assistance, contact Lachat Instruments at 1-800-247-7613, account # 119791.
- 19.4 Lachat Auto Analyzer Data Backup Procedure – Use CDRW on the instrument computer to back up data. This should be done annually, and noted in the instrument maintenance logbook and the data spreadsheet.

## 20.0 Contingencies for Handling Out of Control or Unacceptable Data

- 20.1 Remark Code and Comments – Data and/or samples that do not meet acceptance criteria upon receipt or during analysis need to be either Remark Coded and/or Commented on in the Sample Master LIMS system.
  - 20.1.1 Remark Codes – Remark codes are entered after all data has been parced into Sample Master. To enter a remark code, go to Results Entry. Select QC Batch ID of the data and click Retrieve. In the upper left corner of the View Results window, click on Results to Validate option. Click the “+” box of the sample/QC data needing the remark code and place the appropriate code in the Remark Column. Click the “Close” button on the bottom left of the window (do not click the Validate button) to close the window. All remark codes will be saved. The following codes are to be entered in the appropriate field in Sample Master when necessary:

**Table 4**

SRM Code ID	Description
B	Reported value is associated with a blank contamination
BH	Reported value may be biased high

BL	Reported value may be biased low
D	Dilution resulted in instrument concentration below PQL
E	Estimated Value
H	Hold time exceeded
I	Matrix Interference
N	Not processed
O	Outside calibration range, estimated value
OL	Outside Limit

**20.1.2** Comments – Additional analyst comments can be entered when more detail information is needed. To enter a comment, go to Results Entry. Select QC Batch ID of the data and click Retrieve. In the upper left corner of the View Results window, click on Results to Validate option. Click the “C” box of the sample/QC data needing the comment to open the Comment window. Enter the comment into the field and click OK when finished. Click the “Close” button on the bottom left of the window (do not click the Validate button) to close the window. All comments will be saved.

**20.2** Method Blank contamination – Acceptance criteria for Method Blanks  $\leq \frac{1}{2}$  the PQL. Samples must be bracketed by passing Method Blanks to report samples at the PQL. If a Method Blank fails  $> \frac{1}{2}$  the PQL, the PQL will be adjusted and remark coded “BH” (section 20.1.1) for samples with results between the 5 – 10ppb. Samples with results  $> 10$ ppb within the same bracket will not need a remark code.

**20.3** Duplicate – RPD acceptance criteria for Duplicate analysis  $\leq 15\%$  for samples  $> \frac{1}{2}$  the PQL. The sample result for the Duplicate sample is remark coded “OL” (section 20.1.1) if the Duplicate RPD is  $\geq 15\%$ . If a Duplicate RPD fails, the sample result of the Duplicate is remark coded “OL” (section 20.1.1). If the sample result  $\leq \frac{1}{2}$  the PQL, the Duplicate RPD does not need to be remark coded.

**20.4** MS – MS recovery acceptance criteria  $\pm 15\%$ . The MS result is remark coded “OL” (section 20.1.1) if the MS recovery is  $\geq 15\%$ .

## **21.0 Waste Management**

**21.1** Phosphorus and Dissolved Phosphorus are first digested in individual sample collection vessels using Ammonium Persulfate and Sulfuric Acid. The automated analysis system utilizes several reagents containing toxics and corrosives such as Ammonium Molybdate, Ammonium Persulfate, and Sulfuric Acid. Because of their toxic and corrosive characteristics, sample analysis waste is collected into a 20 liter waste container and disposed through ESF. A “waste tag” is filled out by the employee generating waste, and submitted to the LSO who then enters tag information on line, for pickup.

**21.2** Because the sample is digested in each individual sample collection vessel, the unused sample (in this case, digestate) is poured into analysis waste container and disposed through ESF in the manner stated above.

- 21.2 See Appendix II of Department of Environmental Conservation Laboratory Final Laboratory Waste Management Plan, September 3, 2012.

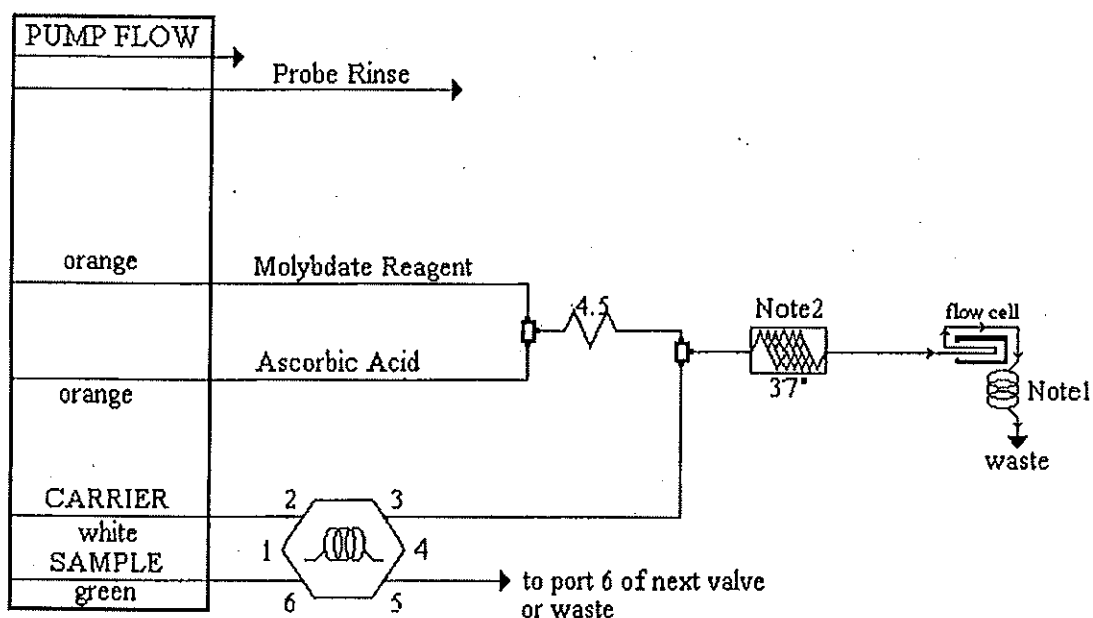
**22.0 References**

- 22.1 Standard Methods for the Examination of Water and Wastewater, 21<sup>th</sup> Ed. APHA-AWNA/WPCF Method 4500-P H Manual Digestion and Flow Injection Analysis for Total Phosphorus.
- 22.2 Determination of Total Phosphorus by Flow Injection Analysis Colorimetry, QuikChem Method 10-115-01-1-F, 27Aug03/csv.
- 22.3 QuikChem FIA+ Automated Ion Analyzer User Manual, Lachat Instruments, Hach Company, 2003.
- 22.4 Omnion 3.0 Software User Guide, Lachat Instruments, Hach Company, 2003.


## 23.0 Tables, Diagrams, Flowcharts and Validation Data

### APPENDIX 1

Total Phosphorus Manifold Diagram



**Carrier:** 0.13 M sulfuric acid  
**Manifold Tubing:** 0.8 mm (0.032 in) i.d. This is 5.2  $\mu$ L/cm.  
**AE Sample Loop:** 100 cm  
**QC8000 Sample Loop:** 100 cm  
**Interference Filter:** 880 nm

**Apparatus:** An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at the specified temperature.

**4.5:** 70 cm of tubing on a 4.5 cm coil support

**Note 1:** 200 cm back pressure loop, 0.52 mm (0.022 in.) i.d.

**Note 2:** 175 cm of 0.8 mm i.d. tubing on the heater.

## Appendix 2

### 23.1 Data System Parameters for Quikchem 8000

Under **Analyte** tab, Channel 1

Property	Value
Description	Phosphorus-Digested
Channel OFF	(box not checked)
Method	FIA

Under **Analyte** tab, Channel 1, Phosphorus-Digested

Property	Value
Analyte Name	Phosphorus-Digested
Concentration Units	ug/l
Calibration Fit Type	Second Order
Clear Calibration	Yes
Force Through Zero	No
Calibration Weighting	None
Auto Dilution Trigger	Yes
% of High Standard	100
Quick Chem Method	10-115-01-1-F
Chemistry:	Direct/Bipolar
Calibration by Height	No

Under **Timing** tab, Run

Property	Value
Method Cycle Period(s)	60
Sample Period (s)	23
Min. Probe in Wash Period	10
Pump standby active	Yes
Use minutes	No
Channel in minutes	No
Analyte in minutes	No
Pump idle before standby	0
Pump at speed before analysis	0

Under **Timing** tab, Run, Channel 1

Property	Value
Load period (s)	18
Inject period (s)	42
Time to valve (s)	26
Use retention time	No

Under **Timing** tab, Run, Channel 1, Phosphorus-Digested

Property	Value
Expected inject to peak start (s)	6
Expected peak base width (s)	65

Under **Rack** tab, choose 3x60 rack configuration

Under **Run** tab, the drop down boxes should show the following

**Instrument:** Instrument 1 (Flow Injection Analysis)  
**Autosampler:** Autosampler 1 (ASX 500 / DRD)

Data configuration for export are set from tool bar. Go to *Configuration* and open *Options*. Choose **Data Export** tab. Under *File Export*, the following boxes should be checked

Export to CSV File Enabled

Include Column Headers

Export in Omnion 2.0 Format

Click on *Data Items*. This will open *Export Data* window. The following items should be entered in the following order. The order is important.

**Location** (to export) F:\ drive

For Channel Independent Data, the Selected Items are (in order shown)

Sample ID  
 Sample Type  
 Replicate Number  
 Detection Date  
 User Name  
 Detection Time  
 Manual Dilution Factor  
 Auto Dilution Factor

For Channel Dependent Data, the Selected Items are (in order shown)

Channel Number  
 Analyte Name  
 Peak Concentration  
 Concentration Units

**24.0 Additional Notes:**

<b>Date:</b>	<b>Revision #:</b>	<b>Summary of Changes:</b>	<b>Submitted By/Date:</b>	<b>Approved By:</b>	<b>Effective Date:</b>
1/28/2015	7	This SOP was rewritten to include NELAC required format and informational points. Where guidance is not applicable or would be redundant, an N/A, or another section within the SOP is referenced.	DGM/CMB 1/28/2015		

## Standard Operating Procedure Signature Page

The signatures below indicate the analyst has read, understands, and will follow the SOP.

[illegible]