ANALYSIS OF LOW-LEVEL TOTAL PHOSPHORUS USING THE SEAL SEGMENTED FLOW ANALYZER

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1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of total phosphorous (TP) in drinking, surface, saline waters, and wastewaters. Higher concentrations may be measured by using sample dilution.

Calibration range	0 – 0.10 mg P/L
Method Detection Limit (MDL)	0.002 mg P/L
Practical Quantitation Limit (PQL)	0.005 mg P/L

1.2. The tests covered by this SOP are W-S-A-TP and W-S-A-TP-F.

2. SUMMARY OF THE METHOD

2.1. The described procedure is based on EPA Method 365.1, Rev. 2.0 (1993) and Seal Method G-146-95 Rev. 3, and the Seal AutoAnalyzer 500 method A-036-19 Rev. 1.

2.2. Prior to analysis samples are prepared by autoclave digestion (DEP SOP NU-049) in which all phosphate containing compounds, both organic and inorganic, are hydrolyzed to generate orthophosphate ion (PO_4^{3-}). During analysis orthophosphate forms a complex with molybdenum and antimony in an acid medium. This phosphoantimony/molybdenum complex is reduced with ascorbic acid and generates a blue colored solution. The intensity of this color is measured at 880 nm for total phosphate analysis.

2.3. Interferences – High iron concentrations can cause precipitation and subsequent loss of phosphorus. High conductivity can cause interferences in matrix spike recoveries.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – SEAL Analytical Segmented Flow Analyzer Systems

Instrument Unique ID	Component	Manufacturer	Model	Application Notes
Bran Luebbe B	Autosampler	SEAL Analytical	XYZ-3	sample time 10/wash time 30
	Pump	SEAL Analytical	AA3 Pump 4	Red switch: Run Pump speed (black switch): Normal
	Manifold	SEAL Analytical	AA3	Heater Assembly at 37°C
	Colorimetric Detector	SEAL Analytical	AA3	Flow cell: 10 mm (Size can be changed) Filter: 880 nm
	Software	SEAL Analytical	AACE 7.10	
Bran Luebbe E	**Autosampler**	**SEAL Analytical**	**AS-4**	**sample time** 10**/wash time 3**0
	Pump	SEAL Analytical	AA500	Pump speed: Normal
	Manifold	SEAL Analytical	AA500	Heater Assembly at 37°C
	Colorimetric Detector	SEAL Analytical	AA500	Flowcell: 10 mm, Filter: 880 nm
	Software	SEAL Analytical	AACE 8.02	

3.2. Equipment –

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Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher Scientific	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher Scientific	Research Plus, 3123000080
Balance, Analytical	Mettler Toledo	MS204TS/00
Volumetric Flask, Class A, Assorted	Fisher Scientific (SR)	10-208M, 10-212-5L, 10-212E, 10-212B
Stir Bar	Fisher Scientific (SR)	14-511-65
Spatula	Fisher Scientific	14-357Q
Stirrer	Corning Stirrer	Scholar 171
Graduated Cylinder, Class A	Fisher Scientific (SR)	08-548B, 08-553A
Volumetric Pipette, Class A, Assorted	Fisher (SR)	13-690-110; 13-681-50
Glass Beaker, Assorted	Fisher Scientific (SR)	02-555-20E, 02-540K, 02-540E
Or Equivalent **[1]Note: (SR) designates equipment is typically ordered through the in-house stockroom**		

3.3. Supplies/Consumables –

Supplies/Consumables	Vendor[1]	Catalog Number
Pump Tubing, ORN/WHT (0.23 mL/min)	Pulse Instrumentation	170-0596-06
Pump Tubing, BLK/BLK (0.32 mL/min)	Pulse Instrumentation	170-0596-07
Pump Tubing, RED/RED (0.80 mL/min)	Pulse Instrumentation	170-0596-10
Pump Tubing, GRN/GRN (2.00 mL/min)	Pulse Instrumentation	170-0596-14
Pump Tubing, Airbar	SEAL Analytical	117+0540-07
Polyethylene Tubing (0.03 ID)-Sample Line (AA3/AA500), waste (AA500)	SEAL Analytical	562-2015-01
AS-4 Pump Tubing-AA500 (IN)	SEAL Analytical	168+9420-01
AS-4 Pump Tubing-AA500 (OUT)	SEAL Analytical	168+9422-01
Pump Lubricant and Oil	SEAL Analytical	590-0161-01; 538+9000-01
Pump and Autosampler Cleaning Swabs	SEAL Analytical	AQCSW-20
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher (SR)	05-403-116
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Sample Cups, Polystyrene, 4mL	Fisher (SR)	02-544-4
Standard Rack Tubes, 16 x 100 mm	Fisher	14-962-10D
Centrifuge Tubes, Polypropylene, 15 mL	Fisher (SR)	05-538-59A
Syringe, Polypropylene, Assorted	Fisher (SR)	22-204-500; B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-102; 02-202-100
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Reagent Bottles, Polyethylene, Assorted	Fisher (SR)	05-716-151; 05-719-146; 05-719-145; 05-719-88
Reagent Bottles, Amber Glass, Assorted	Fisher (SR)	05-719-75; 05-719-183
Parafilm	Fisher (SR)	13-374-12
Or Equivalent **[1]Note:** (SR) designates supply is typically ordered through the in-house stockroom		

3.3.1. Pump Tubing –

Orange/White Sample

Orange/White Ascorbic Acid

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Orange/White	Sulfuric Acid Reagent
Black/Black	Air
Red/Red	Working Color Reagent
Green/Green	Wash (AA3)

Note: For the AA500, the wash is pumped through a separate pump called the AS-4 pump, rather than the main instrument pump. Except for the AS-4 pump tubing, the straws and long tubing are typically not replaced by the analyst.

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 AND NU-042)

Note: Refer to DEP SOP NU-049 for the preparation of all standard solutions.

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water, if needed. Before preparing reagents, standards, or running samples, please read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

Note: All reagent and standard preparations must be documented in the LIMS 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the reagent and standard log sheet (NU-RGTSTD) submitted with each analysis folder. If available, a Certificate of Analysis (CoA) for each purchased reagent, standard, or chemical is maintained in the Analysis folder on the Nutrients drive at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TotalP\CertificatesofAnalysis\. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1. Chemicals –

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Storage
4.1.1.	Ammonium Molybdate Tetrahydrate	$H_{24}Mo_7N_6O_{24}$ *$4H_2O$	Fisher Chemical	A674-500	ACS, 81.0 – 83.0%	Room Temp
4.1.2.	Antimony Potassium Tartrate	$K(SbO)C_4H_4O_6$*$1/2H_2O$	Fisher Chemical	A867-250	USP, 99.0 – 103.0 %	Room Temp
4.1.3.	L-Ascorbic Acid	$C_6H_8O_6$	Fisher Chemical (SR)	A61-100	ACS, ≥99%	Room Temp
4.1.4.	Sodium Dodecylsulfate	SDS	Fisher Chemical	O2674-25	ACS, ≥95%	Room Temp
4.1.5.	Sulfuric Acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus ≥95%	Room Temp
4.1.6.	Bleach, Generic	NaOCl, For cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	Fridge
4.1.7.	Hydrochloric Acid	HCl	Fisher Chemical (SR)	A144S-212	Concentrated, ACS Plus	Room Temp
4.1.8	Sodium Hydroxide	NaOH	Fisher Chemical (SR)	S318-3	ACS, ≥97%	Room Temp
4.1.9.	Deionized (DI) Water	H_2O	In house	N/A	N/A	N/A

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Or Equivalent
[1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom

Deionized water is replaced daily. All other purchased chemicals are given the expiration date from the CoA and/or chemical container. If there is not an expiration date listed, the expiration date is designated by following the protocol in SOPs NU-041 and NU-042.

4.2. Reagents:

4.2.1. Color Reagent –

Antimony Potassium Tartrate (4.1.2.)	0.10 g
Ammonium Molybdate (4.1.1.)	2.4 g
Sulfuric Acid (4.1.5.)	22 mL
Sodium Dodecylsulfate (4.1.4.)	1.4 g
DI Water (4.1.9.)	to 1000 mL

To a 1 L volumetric flask containing approximately 500 mL DI water, first add 0.10 g of antimony potassium tartrate, followed by 2.4 g ammonium molybdate. Slowly, while swirling, cautiously add 22 mL of concentrated H_2SO_4. Cool flask in a water bath if needed. Add 1.4 g of SDS and mix well. The order in which the reagents are added is important. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in a refrigerator using an airtight amber container. Holding time, 5 days.

4.2.2. Sulfuric acid reagent –

Sulfuric Acid (4.1.5.)	5.5 mL
Sodium Dodecylsulfate (4.1.4.)	0.8 g
DI Water (4.1.9.)	to 500 mL

To a 500 mL volumetric flask containing approx. 250 mL DI water add 5.5 mL of concentrated H_2SO_4 and 0.8 g of SDS. Bring to volume with DI water and invert several times to mix thoroughly.

Store in the refrigerator. Holding time, 5 days.

4.2.3. Ascorbic acid reagent –

L-Ascorbic Acid (4.1.3.)	5.5 g
DI Water (4.1.9.)	to 500 mL

To a 500 mL volumetric flask containing approx. 250 mL DI water add 5.5 g ascorbic acid. Bring to volume with DI water and invert several times to mix thoroughly.

Store in a dark bottle in the refrigerator. Holding time, 5 days.

4.2.4. Bleach Cleaning Solution –

Bleach (4.1.6.)	10 mL
DI Water (4.1.9.)	to 100 mL

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Prepare a 10% v/v bleach solution by adding 10 mL Bleach to approx. 50 mL DI water. Dilute to volume and mix thoroughly.

Do NOT use bleach with additives (including Clorox brand).

The bleach solution is made fresh for each use.

4.2.5. SDS wash –

Sodium Dodecylsulfate (4.1.4.)	2 g
DI Water (4.1.9.)	1000 mL

To a 1 L flask containing approx. 600 mL DI water, add 2 g of SDS. Dilute to volume and invert several times to mix thoroughly.

Store at room temperature. Holding time, 180 days.

Note: Carrier solution (see Section 6.1.5.) is digested for use in this analysis (see DEP SOP NU-049, Section 4.2.4.). Document the unique ID and expiration date of the carrier solution on the reagent and standard logsheet in the analysis folder.

4.3. Calibration Standards and Quality Control Standard – Refer to DEP SOP NU-049 for the preparation of all standard solutions. The prepared ALTQC (designated as ERAQC#) is diluted 1:10 for this analysis.

5. SAMPLE PREPARATION, PRESERVATION, AND HANDLING

5.1. Refer to DEP SOP NU-049 for sample preservation, holding time, preparation instructions and sample handling details. The carrier solution, samples, QC standards, and blanks are digested prior to analysis.

6. SAMPLE ANALYSIS/QUANTITATION/CALIBRATION

Note: Read the manufacturer operation manual before proceeding. Follow DEP SOP NU-033.

6.1. Detector and Pump Start-Up Procedure:

6.1.1. AA3: Turn on the instrument computer. Next, turn on the autosampler and allow needle to return home. Then turn on the AA3 pump followed by the AA3 Digital Colorimetric detector (heater will take approximately 15 minutes to come to temperature). Then secure platens for the tubing being used.

AA500: Turn on the instrument computer. Next, turn on the autosampler and allow needle to return home. Turn on master switch for the AA500 system. Platen is secured by holding the platen button until the platen stops.

6.1.2. Double click on the AACE icon on the desktop to start the main program.

6.1.3 AA3: Click on the “Charting” icon and make sure that the pump’s two rocker switches are set to Run and Normal (speed) respectively. Close out Channel 2, since only Channel 1 will be used for the run.

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AA500: Click on the "Charting" icon. To start the pump, press the on/off button. There is a fast button if needed. Close out Channel 2, since only Channel 1 will be used for the run.

Note: Ensure the correct method is selected upon charting. For Total-Phosphorus, the method is "FDEP LLTP".

6.1.3. Perform the daily wash – Flush the lines with DI water for 10 to 15 min until the baseline is stable (normally 5%).

Alternatively, the lines are flushed with the following reagents to maintain a stable baseline. The analyst may decide whether this step needs to be performed depending on the baseline noise and the number of samples analyzed daily. This step is typically performed every other day. The lines are flushed sequentially as follows:

a. 1:10 Hydrochloric acid (5 minutes)
b. DI water (5 minutes)
c. 1 N Sodium Hydroxide (5 minutes)
d. DI water (10 – 15 minutes)
e. SDS wash (see Section 4.2.5.) for 15 minutes.

The system is also flushed with 10% v/v bleach solution (4.2.4.) once a month or if contamination is suspected. Pump tubing must be replaced after a bleach wash. The bleach solution must not touch the autosampler needle, as it will cause damage.

6.1.4. While proceeding with the wash procedure (6.1.3.), type up the run. Click on Set Up → Analysis → LLTP → New Run → Tray Protocol and save the new run as "TPYYMMDDX#". The file name follows the TPYYMMDDB# data format, where TP stands for Total Phosphorus, YY is the year, MM is the month, DD is the day of the month, and X indicates the B+L instrument used for the run (B or E). Postscripts 1, 2 etc. are used to denote the first or second run of the day. For example, the first run on Sept. 1, 2015 would be named TP150901B1.

Then using the prep worksheet (see DEP SOP NU-049, Section 5.7.), begin typing up the run, in order of the batches listed on the worksheet. Type the tray number beside the PQCK1 at cup #18 (e.g. PQCK1_TP3287), and the prep batch ID number with each MBLK sample line. Also enter the SRM name with no spaces between characters but a space for the dilution factor (e.g., for ERAQC17 with a dilution factor of 10, type ERAQC17 10). Print the run; this will be the bench sheet for the run.

An example run sequence includes:

Baseline
Primer – the B+L high standard (see DEP SOP NU-049, Section 4.3.3.)
Drift – the B+L high standard (see DEP SOP NU-049, Section 4.3.3.)

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2 BLKs
Calibrants – (see DEP SOP NU-049, Section 4.3.3.)
High/Low/Low sequence – to check carryover (the B+L high standard followed by two blank cups)
PQCK1 (PQL and MDL verification spike) tray # (See DEP SOP NU-049, Section 4.3.3.)
ERAQC# dilution factor (See DEP SOP NU-049, Section 4.3.6.)
CCB and CHCK1 – these checks bracket every 10 cups on the run (see DEP SOP NU-049, Sections 4.3.8. and 4.2.3.), and may be followed by a Drift cup and a Baseline check
Batched samples, starting with the method blank (MBLKs) for each batch (indicate prep batch ID)
Final CCV/CCB bracket with a Drift cup and a Baseline check (see Section 6.1.8.)

Note: LLTP samples are normally run initially on the discrete analyzer (AQ2 or AQ400, see DEP SOP NU-090). Sample results above 0.05 mg P/L are reported from the AQ2 or AQ400, and samples with concentrations below 0.05 mg P/L are run on the Bran+Luebbe. Check carefully against the backlog or prep worksheet to make sure that no samples are missed in the process. Matrix spikes and laboratory fortified blanks (LFB's) are analyzed on the AQ2 or AQ400.

Note: Multiple trays can be run on the same run sequence using one calibration. Indicate on the report which tray the BLK, CCV, and calibration standards came from. Identify the tray QC in the sequence. The first ALTQC should be identified as the initial calibration verification (ICV). A failing ICV renders the calibration unusable.

6.1.5. Place all reagent tubing in their respective reagent bottles.

- Sulfuric acid reagent (Orange/White tubing)
- Ascorbic acid reagent (Orange/White tubing)
- Working color reagent (Red/Red tubing)

Place the sample wash line (Green/Green tubing for AA3, Polyethylene tubing for AA500) in the digested carrier solution container (see DEP SOP NU-049, Section 4.2.4.). Allow the reagents to run through the tubing for approximately 20-30 minutes, while adjusting the baseline every 5 minutes until the baseline is stable (5.0% – 5.1%). To view the current baseline, click on 'Charting' and check that the baseline is stable and that the complete system is ready. To adjust the baseline, right-click on 'Set Base' and select 'Auto Base' from the pop-up menu (normally 5%).

6.1.6. While waiting for the baseline to stabilize (6.1.5.), pour up the run. Use the printed bench sheet to pour up the QC and samples in order. Use clean 4 mL autosampler cups and label them with QC/sample ID to avoid confusion. Rinse each cup with a small amount of sample before filling the cup and place it in the correct spot on the analysis tray. Remove the old calibration standard solutions from the standards rack on the autosampler and replace them with fresh standards.

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6.1.7. After pouring up the samples and checking the baseline for stability, start the run. Click on Run → Start → LLTP → Current Run (scroll down). Double click on "Current Run" and enter the analyst's initials next to the Operator field followed by the appropriate instrument next to Instrument. Click on "Start". Monitor the run for bad peaks (peak shape, air spikes etc.) and failing QC. Rerun any samples with air spikes or poor peaks at the end of the run.

6.1.8. When you are ready to stop the run, go to Setup → Analysis → LLTP and double click on Run → Tray Protocol. Add the label "END" at the cup number where you wish the run to stop. Make sure that all samples are bracketed by passing CCVs and blanks. There should be a Blank, CCB, CHCK1, Drift and Baseline (in that order) prior to the cup labeled "END".

6.1.9 AA3: After the run is complete, flush the system tubing for 10 minutes with DI water or SDS wash solution (4.2.5.) as needed. Turn the pump off. Release the pump's platen cover to release the pressure on the pump's tubing. The instrument is turned off in the opposite way it was turned on (6.1.1.).

AA500: After the run is complete, run shut down procedure which flushes the tubing with DI water for six minutes on fast before releasing the platen and turning the pump off. Turn the instrument off.

6.2. Quantitation/Calculations:

6.2.1. After completion of the analysis print a summarized analysis report containing the peaks, calibration graph, and values. Also print the raw data. The instrument reports analysis results in mg P/L.

6.2.2. The individual calibration standards (seven plus a calibration blank) are prepared according to Section 4.3.3. in DEP SOP NU-049. The linear calibration curve, including the correlation coefficient and the % error between the target value of each calibrant and its calculated value, is documented by the instrument software and is part of the analysis report.

6.2.3. The QC Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes. For general calculations including RPDs and spike recoveries see the FDEP Quality Manual (Section 5.9.).

6.2.4. Convert the excel file into Raw data in the QC Manager to the appropriate instrument.

7. DATA ARCHIVAL/VALIDATION

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test/s (W-S-A-TP, W-S-A-TP-F), filename/s (TPYYMMDDX#, see Section 6.1.4. for a detailed description of the file naming convention), and

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initials of the analyst. Submit the folder to a supervisor for review and authorization.

7.2. Each report folder should contain the following items for the Bran+Luebbe run – analysis report, bench sheet, raw data sheet(s), digestion temperature log, Reagent and Standard Logsheet, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. Bran+Luebbe instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder of the specific instrument located at, \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\SegmentedFlowAnalyzers\.

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\floridadep\data\labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE SOPS NU-038 AND NU-043)

8.1. For method performance and acceptance criteria for QC measures and contingencies for handling out-of-control or unacceptable data refer to the following DEP SOPs, NU-038 and NU-043.

8.2. A check standard (CHCK1; 0.04 mg P/L; see DEP SOP NU-049, Section 4.3.8.) and CCB (blank) are run at the beginning, every 10 cups, and at the end of each run. Acceptance limits for recovery of the CHCK1 standard are 90 – 110%. Acceptance limits for the MBLK and CCB are > -0.002 and < 0.002 mg P/L.

8.3. A laboratory fortified blank (LFB; 0.4 mg P/L; see DEP SOP NU-049, Section 5.10.) and duplicate samples and/or duplicate matrix spikes are analyzed on the AQ2 instrument (see DEP SOP NU-090) for every batch of 10 samples. Acceptance limits for the LFB and matrix spike recoveries are 90 – 110%. The sample/matrix spike reproducibility should be less than 20% RPD for results above the PQL.

8.4. One practical quantitation limit level standard, PQCK1 (0.005 mg P/L; see DEP SOP NU-049, Section 4.3.3.), is analyzed on every analytical run. Acceptance limits for recovery of the PQCK1 standard are 70 – 130%. The PQCK1 standard also acts as the MDL spike for ongoing MDL and PQL verification. (see DEP SOP LB-031).

8.5. One certified standard reference material, ALTQC, is analyzed on every analytical run. The supplier for the QC stock standard differs from the supplier of the calibration standards. Acceptance limits for recovery of the ALTQC standard are 90 – 110%.

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8.6. Linear Calibration Range (LCR) – See FDEP Quality Manual Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the linear calibration must be equal to or larger than 0.995. Acceptance limits for the percent error of calibration standards above the PQL are ±10%. Acceptance limits for the percent error of the PQL level standard is ±30%. Acceptance limits for the percent error of the 0.003 mg P/L calibrant are -33 to 50%. The acceptance limits for the blank calibration point result are below the MDL and above the negative value of the MDL (-MDL < blank < MDL). Every day samples are analyzed a new calibration must be performed and verified at the beginning of the analysis. Consecutive runs on one day may use the same calibration.

8.7. Annual MDL and PQL verification is required, (see NU-043, 2.3.7). Method blank (MBLK) and PQCK1 data is collected and utilized for the ongoing MDL and PQL verification. Perform annual verifications using the LIMS statistics tracker module.

8.8. The carryover percentage should be less than 2 %; high carryover indicates the need for instrument maintenance or troubleshooting. If the carryover is above 2%, instrument maintenance should be performed. Samples must be re-analyzed unless a supervisor has evaluated the analytical run and given approval to report the samples.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

9.1. Review the laboratory Health and Safety Plan (accessible at https://floridadep.sharepoint.com/dear/SitePages/Lab Health and Safety.aspx).

9.2. Safety glasses, gloves and lab coats should always be worn handling samples and reagents.

9.3. Read the MSDS information for safe reagent and sample handling.

9.4. Sample waste, excess reagents, and waste exiting the flow cell may be poured down the sink while flushing with water.

10. MANIFOLD CLEANING, MAINTENANCE

10.1. Observe routine maintenance as recommended by instrument manufacturer and enter maintenance performed in the maintenance logbook.

10.2. Flush the instrument on a regular basis as described in Section 6.1.3.

10.3. Change the pump tubing every 3 weeks or more often if needed. For the AA500, replace the AS4 pump tubing as needed.

11. REFERENCES/DEFINITIONS

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

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11.1. EPA method 365.1, Rev. 2.0 (1993).

11.2. Bran+Luebbe™ Analyzer Series AA3 System Operation Manual.

11.3. Bran+Luebbe method for Total Phosphorous G-146-95 Rev.3.

11.4. Quality Manual, Bureau of Laboratories, FDEP.

11.5. Laboratory Health and Safety Plan.

11.6. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

11.7. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

11.8. DEP SOP NU-041, Reagent Preparation Procedure.

11.9. DEP SOP NU-042, Standard Preparation Procedure.

11.10. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

11.11. DEP SOP NU-049, Total Phosphorus (TP) Autoclave Sample Preparation.

11.12. DEP SOP NU-090, Determination of Total Phosphorus in Water using the AQ2 Discrete Analyzer.

11.13. DEP SOP LB-031, Limit of Detection Verification.

11.14. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

Appendix of Significant Changes

02/22/2008	Updated SOP to comply with the latest revision of method: 365.1, *Revision2.0,* August 1993.
02/23/2010	Updated section 6 of the SOP by including greater detail on the daily operation of the instrument.
02/15/2012	Removed the standard preparation section and added it to the sample digestion SOP (NU-049).
09/17/2012	The MDL and calibration range were changed. The MDL is 0.002 mg P/L. The calibration range is from 0.005 mg P/L (PQL) to 0.10 mg P/L.
08/20/2015	Data archival information added to section 7.
12/20/2018	Ascorbic acid reagent preparation description was corrected (4.3.2.). MDL (blank) is discontinued in the run sequence. Ongoing MDL verifications are based on MBLK's and MDL verification spikes. The PQCK1 serves as the MDL and PQL verification spike. The daily wash procedure in section 6.1.3. was modified.
12/10/2019	Section 6 was modified to clarify the procedure for running multiple trays on one run. Section 8 was expanded to include the percent error acceptance limits of the calibration standards. In Section 12 technical support information was removed.

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02/13/2020 Added acceptance limits for the calibration points below PQL.

08/01/2023 Added table of contents. Added SOP tables. Updated data drive file path links.

10/03/2024 Updated data drive file path links.

01/20/2025 Added AA500 instrument.

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