TOTAL PHOSPHORUS (TP) AUTOCLAVE SAMPLE PREPARATION

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1. SCOPE AND APPLICATION

1.1. This method details the preparation of aqueous samples for total phosphorus (TP) determination.

1.2. All other required information about the analysis, such as method detection limits (MDLs), interferences, quality control, data assessments and acceptance criteria for quality control measures, can be found in DEP SOPs NU-082 and NU-090.

2. SUMMARY OF THE METHOD

2.1. This method is based on EPA method 365.1, Rev. 2.0 (1993).

2.2. Both organic and inorganic phosphorus in a sample are converted to orthophosphate through a digestion with sulfuric acid and ammonium persulfate. The total phosphorus is then analyzed as orthophosphate by a colorimetric method (see DEP SOPs NU-082 and NU-090). For a discussion of interferences refer to DEP SOPs NU-082 and NU-090.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation –

Equipment ID	Component	Manufacturer	Model
Tuttnauer1	Autoclave	Tuttnauer	3870 ELP-B/L-D
Consolidated Sterilizer	Autoclave (Backup)	Consolidated Sterilizer	SSR-3A-PB

3.2. Equipment –

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Fixed Volume Pipette, 200 µL	Eppendorf/Fisher Scientific	Research Plus, 3124000083
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher Scientific	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher Scientific	Research Plus, 3123000080
Adjustable Pipette, 2-20 mL	Rainin	Pipet-Lite Pipette LTS L-20MLXLS, 17011788
Repeating Pipetter, 500 µL	Fisher Scientific	HandyStep™ S, 13-668-722
Autoclave Tubes and caps	Environmental Express	EE3457109
Test Tube Racks	Fisher Scientific	14-809C
Balance, Analytical	Mettler Toledo	MS204TS/00
Balance, Top Loading	Mettler Toledo	ARD110
Volumetric Flask, Class A, Assorted	Fisher Scientific (SR)	10-208M, 10-212-5L, 10-212E, 10-240E, 10-212B
Stir Bar	Fisher Scientific (SR)	14-511-65
Spatula	Fisher Scientific	14-357Q
Stirrer	Corning Stirrer	Scholar 171
USB Drive	SanDisk	Cruzer Glide 32GB
Data Logger	MadgeTech	HiTemp140
USB Docking Station for High-Temperature Pressure Data Logger	Fisher Scientific	15-060-081
Bottle, Teflon, 2000 mL	Fisher Scientific	02-923-30E
Bottle, Polyethylene, Assorted	Fisher Scientific (SR)	05-719-88, 05-719-145
Graduated Cylinder, Class A	Fisher Scientific (SR)	08-548D

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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
Pipette tips, Rainin, 2-20 mL	Fisher Scientific (SR)	NC0684183
Pipette tips, Eppendorf, 200 µL	Fisher Scientific (SR)	05-403-41
Pipette tips, Eppendorf, 1000 µL	Fisher Scientific (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher Scientific (SR)	05-403-116
Dispenser Tips, Fisherbrand, 12.5 mL	Fisher Scientific	13-668-707
Sample Beakers, Polystyrene, Assorted	Fisher Scientific (SR)	08-732-119, 08-732-122
Parafilm	Fisher Scientific (SR)	13-374-12
Kimwipes Sm, 4.5 x 8.5''	Fisher Scientific (SR)	06-666A
Pipette, Transfer, Polyethylene, 5 mL	Fisher Scientific (SR)	13-711-5AM
Dish, Weigh, Hexagonal, 4X3	Fisher Scientific (SR)	02-202-102
ChamberBrite	Fisher Scientific	13878000
Or Equivalent		
[1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

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

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 prep worksheet in LIMS. The unique ID and expiration date of the carrier solution (4.2.4) is documented in the analysis folder (see DEP SOP NU-082). If available, a Certificate of Analysis for each purchased reagent, standard or chemical is also maintained in a folder on the Nutrients drive at \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TotalP\CertificatesofAnalysis\Digestion\ . All containers holding standards or reagents must be labeled with their unique IDs and expiration date.

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

Note: Prior to using the chemicals in this analysis, read their MSDS sheet and the safety notes found in Section 8 of this SOP.

4.1. Chemicals –

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Sulfuric Acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Certified ACS Plus	N/A	Room Temp
4.1.2.	Ammonium Persulfate	APS	Fisher Chemical (SR)	BP179-100	≥98.0%, Electrophoresis Grade	N/A	Room Temp
4.1.3.	Potassium Phosphate Monobasic	KH_2PO_4	Fisher Chemical	P285-500	≥99%, ACS	N/A	Room Temp

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4.1.4.	Glycine	$C_2H_5NO_2$	Sigma-Aldrich Corp.	G7126-100G	≥99%, HPLC	N/A	Desiccator
4.1.5.	β-Glycerophosphoric Acid, Disodium Salt, Pentahydrate	$C_3H_7Na_2O_6P \cdot 5H_2O$	Arcos Organics	410991000	98%	N/A	Room Temp
4.1.6.	WatR™ Pollution Complex Nutrients	ALTQC, ERA	Environmental Resource Associates	525	See CoA	See CoA	Fridge
4.1.7.	Deionized (DI) Water	H_2O	In house	N/A	N/A	Daily	N/A
Or Equivalent [1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom							

4.2. Reagents:

4.2.1. Sulfuric Acid Digestion Solution –

Sulfuric Acid (4.1.1.)	620 mL
DI Water (4.1.7.)	to 2000 mL

CAUTION! The mixing of concentrated sulfuric acid with water generates much heat and can result in serious injury if not performed carefully. The dilution needs to be done under a fume hood with proper protective equipment (eye protection, gloves, lab coat). The concentrated acid should be added to water slowly with cooling between each addition.

Pour approx. 1.2 L DI water into a 2 L beaker under the fume hood. Slowly and carefully add 620 mL of concentrated H_2SO_4. Leave under the fume hood to allow the solution to cool down to room temperature. Once at room temperature, transfer the solution to a 2 L volumetric flask, dilute to volume with DI water, cap, and invert several times until the solution is thoroughly mixed. Transfer into a clean 2 L Nalgene bottle and close with the pump dispenser. Label the bottle.

Store at room temperature. Holding time, 90 days.

4.2.2. Ammonium Persulfate Solution –

Ammonium Persulfate (4.1.2.)	30 g
DI Water (4.1.7.)	60 mL

Dissolve 30 g ammonium persulfate in 60 mL DI water; mix well until all the persulfate has dissolved. Prepare volume of reagent as needed. Do not change the persulfate to water ratio.

This solution is prepared daily before use when samples are digested. The holding time in LIMS, however; is 30 days in order for digested carrier solution (4.2.4) to also have a 30-day holding time.

4.2.3. Diluent Solution, 0.2% H_2SO_4 –

Sulfuric Acid (4.1.1.)	4 mL
DI Water (4.1.7.)	to 2000 mL

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Add 4 mL of reagent grade sulfuric acid to a 2 L volumetric flask containing approx. 1 L of DI water. Dilute to volume with DI water and invert several times to mix.

Store at room temperature. Holding time, 30 days.

Note: This solution is used for method blanks (MBLKs), laboratory fortified blanks (LFBs), blanks (MDLs, continuing calibration blanks (CCBs)), and sample dilutions.

4.2.4. Carrier Solution (used for Bran+Luebbe (B+L) analyses only) –

Ammonium Persulfate Solution (4.2.2.)	40 mL
Sulfuric Acid Digestion Solution (4.2.1.)	40 mL
DI Water (4.1.7.)	to 2000 mL

Add 40 mL of ammonium persulfate digestion solution and 40 mL of sulfuric acid digestion solution to a 2 L volumetric flask containing approx. 1 L of DI water. Dilute to volume with DI water and mix thoroughly. Transfer the solution to a 2 L Nalgene bottle, cap loosely, and autoclave. Add carrier solution to the digestion sheet. Extra labels with the unique ID and expiration date may be printed for documentation in the analysis folder.

Store at room temperature. Holding time, 30 days.

4.3. Standards and Spiking Solution:

4.3.1. TP/Orthophosphate 1000 mg P/L stock standard –

Potassium Phosphate Monobasic (4.1.3.)	4.394 g
DI Water (4.1.7.)	to 1000 mL

Dry at least 5 g of KH_2PO_4 at 105 °C for 12-24 hours. Allow to cool to room temperature in a desiccator before weighing.

To a 1 L volumetric flask containing approx. 400 mL DI water, add 4.394 g of the dried potassium phosphate. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator Holding time, 180 days.

Potassium Phosphate Monobasic molecular weight factor: 0.2276

4.3.2. TP/Orthophosphate 10 mg P/L stock standard –

1000 mg P/L Stock (4.3.1.)	2.5 mL
DI Water (4.1.7.)	to 250 mL

Into a 250 mL volumetric flask containing approx. 100 mL DI water, pipette 2.5 mL of the 1000 mg P/L stock standard. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

4.3.3. TP working calibration standards –

1000 mg P/L Stock (4.3.1.)	see table

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10 mg P/L Stock (4.3.2.)	see table
Sulfuric Acid (4.1.1.)	see table
DI Water (4.1.7.)	see table

Prepare standards by pipetting the amounts of the 1000 mg P/L stock standard or 10 mg P/L working standard specified in the table below into volumetric flasks filled approximately halfway with DI water. Add the amount of conc. sulfuric acid specified in the table to each flask and dilute to volume with DI water. Invert several times to mix thoroughly. This makes the acid content of the standards the same as that of the preserved samples (0.2%). The following standard solutions must be digested with each tray of samples.

Store in refrigerator. Holding Time, 30 days.

Calibration Standard	Concentration (mg P/L)	QC Standard, Alias	Final Volume	Volume of H_2SO_4 added	Vol of 10 mg P/L standard added	Vol of 1000 mg P/L standard added
BL CAL2	0.003		500 mL	1 mL	150 µL	N/A
BL CAL3	0.005	BL CAL3, BL PQCK1	1000 mL	2 mL	500 µL	N/A
BL CAL4	0.008		500 mL	1 mL	400 µL	N/A
BL CAL5	0.01		500 mL	1 mL	500 µL	N/A
BL CAL6	0.02		500 mL	1 mL	1.0 mL	N/A
BL CAL7	0.05	BL CAL7, AQ PQCK2	1000 mL	2 mL	5.0 mL	N/A
BL CAL8	0.10	BL high standard	500 mL	1 mL	5.0 mL	N/A
AQ2 CAL STD	1.0	AQ high standard	500 mL	1 mL	N/A	500 µL

4.3.4. AQ2/AQ400 Check Standard (CCV; 0.40 mg P/L) –

1000 mg P/L Stock (4.3.1.)	200 µL
Sulfuric Acid (4.1.1.)	1 mL
DI Water (4.1.7.)	to 500 mL

In a 500 mL volumetric flask containing approx. 250 mL of DI water, add 1 mL conc. H_2SO_4. Pipette 200 µL of the 1000 mg P/L TP/PO4 standard, dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

4.3.5. B+L Check Standard (CHCK1; 0.04 mg P/L) –

10 mg P/L Standard (4.3.2.)	2 mL
Sulfuric Acid (4.1.1.)	1 mL
DI Water (4.1.7.)	to 500 mL

In a 500 mL volumetric flask containing approx. 250 mL of DI water, add 1 mL conc. H_2SO_4. Pipette 2 mL of the 10 mg P/L TP/PO4 standard, dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

4.3.6. TP Spiking Stock Solution (50 mg P/L) –

β-glycerophosphoric acid (4.1.5.)	0.4941 g

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DI water (4.1.7.)	to 1000 mL

Add 0.4941 g β-glycerophosphoric acid to a 1 L volumetric flask containing approx. 400 mL DI water. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

Note: For convenience, a combined TKN/TP spiking solution (TKN: 225 mg N/L; TP 50 mg P/L) can be prepared by drying at least 2 g of glycine (4.1.4.) overnight at 60 °C, cooling it to room temperature in a desiccator, and then adding 1.206 g to the flask prior to bringing the standard to volume (see DEP SOP NU-091 for details). If a combined spiking standard is made, this standard should be named "TKN/TP ORG SPIKE" in the Standard Prep Tracker.

Note: This solution is used to prepare LFBs, matrix spikes (MSs), and matrix spike duplicates (MSDs). The combined TKN/TP spiking solution is also used for the analysis TKN in water (DEP SOP NU-092).

Glycine molecular weight factor: 0.1866

β-glycerophosphoric acid molecular weight factor: 0.1012

4.3.7. ALTQC Stock Standard (ERAQC# Stock) –

WatR™ Pollution Complex Nutrients (4.1.6.)	see ERA's instructions
DI Water (4.1.7.)	see ERA's instructions

The quality control standard is prepared from the ampule according to the manufacturer's instructions. The supplier (Environmental Resource Associates) differs from the supplier of the calibration standards. For the concentration and expiration date of the ampule please see the manufacturer's certificate.

Store in refrigerator. Holding time stock standard, 90 days.

4.3.8. ALTQC Working Standard (ERAQC# Working) –

ALTQC Stock Standard (4.3.7.)	refer to certified value
Sulfuric Acid (4.1.1.)	2 mL
DI Water (4.1.7.)	to 1000 mL

In a 1 L volumetric flask containing approx. 500 mL of DI water, add enough of the ALTQC Stock Standard so that the concentration of the working standard falls within the calibration range. Add 2 mL sulfuric acid to acidify to 0.2%. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time working standard, 30 days.

Note: In cases where the certified value of the quality control standard (4.1.6.) falls within the calibration range, further dilution is not necessary. However, the prepared ALTQC MUST be acidified to 0.2% H_2SO_4 and would be considered the ALTQC Working Standard (4.3.8.).

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5. SAMPLE PREPARATION PROCEDURE (SEE DEP SOP NU-033)

5.1. Sample preservation and holding times: Samples are preserved with 0.2% sulfuric acid at collection. They are stored refrigerated (≤ 6 ºC, not frozen) in the custody room C301. The samples have a 28-day holding time.

5.2. Fresh and ground water samples may be batched together. Effluent samples are batched separately. A batch may contain up to 10 samples.

5.3. All samples having conductivities above 5000 µmhos/cm (equivalent to about 3.0 PPTh salinity) are batched separately. They are diluted two-fold prior to digestion unless notified otherwise by a supervisor. (Check the conductivity of the samples in your backlog. If a sample conductivity exceeds 5000 µmhos/cm or salinity of 3 PPTh, it should be digested in a salt batch even if it is not labeled as a salt matrix.)

5.4 Each prep batch contains one MBLK, one LFB, and one spiked sample. See Section 7 for details.

5.5. Samples with TP concentrations above 5 mg P/L must be prediluted to a concentration of less than 5 mg P/L and re-digested.

5.6. Check the backlog prior to preparation. If there are any re-digests, add them to your tray for that day.

5.7. Log the samples out following the proper protocol to preserve chain of custody and allow samples to come to room temperature before digestion. Arrange samples in batch order.

5.8. Remove the ALTQC, the spiking solution, and the calibration standards from the refrigerator and allow them to come to room temperature.

5.9. Open the Prep Worksheet Manager module in LIMS and choose the first icon (which looks like a blank page) to create a new worksheet. Choose “365.1 (March 1983)” from the list of available preparation IDs, select the sample IDs to be added to the worksheet, assign the worksheet to the correct analyst, and click the checkmark to create the worksheet. The worksheet ID generated in LIMS will be the tray number for the digestion. Complete the header information for the sheet, SOP (NU-049), prep analyst name, equipment IDs (e.g., Tuttnauer 1 and all pipettes), and the spike standard solution serial number and reagent serial numbers (ensure that the corresponding identities and expiration dates are correct).

5.10. Update the sample table, beginning with the starter rows for QC samples digested on the tray, ensure that serial numbers, identities, and expiration dates for the standard solutions used are correct. A single prep batch ID will be generated automatically for every sample on the sheet; therefore, assign other prep batch IDs, as necessary, by ‘Control+Clicking’ the sample IDs to be batched together and generating a new prep batch ID for each batch. For the matrix spike sample in each batch, duplicate its row in the sample table **twice** by right-clicking on the row and selecting “Duplicate Row”, then add the spike code ‘_L3_’ and the letter A or B as a suffix to the sample ID. Save the updates by clicking the “Save” button at the bottom of the worksheet.

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Note: To prevent transcription errors, use a barcode scanner for standard/reagent entries when feasible.

5.11. If a sample is re-digested for confirmation the bottle's label must be checked against the field label. Add a comment in the worksheet confirming that the label was checked.

5.12. Select clean tubes for the digestion (see DEP SOP NU-046 for glassware cleaning). Print the tube labels on a Sato printer using the Print Prep Labels module in LIMS, adding the tray ID in the "Date" column of the label. Label tubes accordingly.

5.13. Invert each sample several times to make sure all particulate matter is suspended, and the sample is well mixed. Pour sample into a 50 mL sample beaker and pipet 25 mL into the respective digestion tubes. MBLKs (4.2.3), LFBs (5.13), check standards (4.3.4 & 4.3.5), ALTQC (4.3.8), and calibration standards (4.3.3) are prepared the same way. Four extra tubes with diluent solution are also prepared as blanks. These are used as CCBs. If a sample needs to be diluted, use the following table for dilutions made directly in the tube.

Dilution	Pipette Sample Volume (mL)	Pipette Diluent Volume (mL)	Total Volume in Tube (mL)
1:2	12.5	12.5	25
1:5	5.00	20.0	25
1:10	2.50	22.5	25
1:25	1.00	24.0	25
1:50	0.50	24.5	25

Dilutions that are 100 times or greater must be made via volumetric flask, then added to the tube. Add a note in the sample comments section on the digestion worksheet with the exact dilution made in the volumetric flask.

5.14. Spiking procedure – The spike level is 0.4 mg P/L. LFBs, MSs, and MSDs are prepared by adding 200 µL of 50 mg P/L spiking solution (4.3.6.) to a 25 mL aliquot of sample for MSs and MSDs or to a 25 mL aliquot of diluent solution (4.2.3.) for LFBs.

5.15. Add 500 µL of ammonium persulfate solution (4.2.2.) and 500 µL of the sulfuric acid digestion solution (4.2.1.) to each tube.

5.16. Cap the digestion tubes loosely (1-2 turns) and place the tubes in the autoclave.

5.17. The Tuttnauer autoclave reservoir must be fully drained and refilled with fresh DI water weekly. Add fresh DI water to the autoclave daily if the level on the dipstick is low.

5.18. One spot in the tray is chosen at random to place the data logger (see 3.2.). The data logger is used for temperature recording during the autoclave process. The data logger records temperature readings throughout the entire cycle.

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The data logger program is started in advance using the Fisher Scientific Datalogger software, as follows:

5.18.1. Plug data logger into the USB cable at the PC with the Fisher Scientific Datalogger software installed.

5.18.2. Open the Fisher Scientific Datalogger software. The data logger ID will appear in the "Connected Devices" panel of the software interface. Click on the ID once to highlight it.

5.18.3. In the Device tab menu bar, click Quick Start (this will use the most recent device settings, visible in the Connected Devices panel). When the data logger has been properly started, the status will read "Running".

5.18.4. Unplug the data logger from the USB interface cable and place it in one spot of the autoclave sample tray.

5.19. Press and hold door closed for 10 seconds. The autoclave will lock securely. An error message will appear if closed incorrectly. Select the "TP digestion" cycle. This cycle is preset to 121 °C for 50 minutes.

5.20. Place the ALTQC, spiking, check standard, and calibration standard solutions back into the refrigerator. Return samples to receiving room.

5.21. Once the digestion is complete, remove the tubes from the autoclave and allow them to cool to room temperature. In the rare occasion that a tube is missing a cap, the sample must be re-digested. If a QC tube is missing a cap, consult a supervisor.

Note: **The Tuttnauer autoclave will not open if the temperature is above 90 °C or if the pressure gauge is above zero!** Autoclave contains steam under pressure. Release of pressurized steam could cause injury if autoclave opened too soon.

Note: The Consolidated Sterilizer Autoclave may serve as a backup autoclave. The autoclave must be turned on and the jacket pressure must reach 20 psi. The chamber can then be opened and trays inserted. A data logger must be used to collect temperature readings throughout the cycle. Close the chamber door securely. Adjust temperature and time if needed. The cycle should be 121 °C for 45 minutes. Press the start button. The cycle time begins once the Chamber pressure reaches 20 psi. The autoclave will beep when the cycle is complete. Press the start button to stop. Open the chamber and remove trays. Close the chamber securely and turn the autoclave off.

5.22. Once the cycle is completed, open the door using the up arrow. Remove the data logger from the tray, and repeat steps 5.18.1 and 5.18.2., above. Click "Stop" in the Device tab. Click "Download" to open the temperature report. Close the Fisher Scientific Datalogger software and recharge the data logger battery if necessary.

Use the worksheet ID/tray number (see Section 5.8.) to name the temperature file. Save the report at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TotalP\DataLoggerFiles. Print the report for the analyst to include with the run

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folder. Check carefully to make sure 120 ºC was reached and maintained for 30 min. Acknowledge the acceptability with a check mark (✓) and initials. The temperature report is included in the analysis folder.

5.23. Finish filling out the prep worksheet in the Prep Worksheet Manager module in LIMS:

5.23.1. Open the created worksheet by clicking the spreadsheet icon in the Prep Worksheet Manager module and double-clicking the appropriate worksheet ID from the list. Complete the prep start date/time and prep completion date/time. The prep start time should be reflected by the initial time on the datalogger upon activation, while the completion time should be reflected by the time of the final data point that the datalogger obtains. Double check all other header information.

6. SAMPLE ANALYSIS/QUANTITATION

See DEP SOPs NU-082 and NU-090 for the sample analysis procedure and quantitation information.

7. QUALITY CONTROL/DATA ANALYSIS (SEE DEP SOPS NU-038 AND NU-043)

Note: For method performance and for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to the following DEP SOPS; NU-038, NU-043, NU-082, and NU-090.

7.1. One digested PQL for analysis on the B+L and one for the AQ2/AQ400 per tray.

7.2. One digested ALTQC per tray for analysis on the AQ2/AQ400 and B+L (diluted into the calibration range on the analytical run).

7.3. One digested LFB per batch of 10 samples or less spiked at 0.40 mg P/L.

7.4. One method blank (MBLK) per batch, and 4 extra tubes with diluent solution (4.2.3.) digested for CCBs, blank standards, and sample dilutions.

7.5. One sample spiked per batch at 0.40 mg P/L. This spiked sample is digested in duplicate or a sample duplicate is digested to obtain precision for the batch.

7.6. One digested check standard (CCV) for the AQ2/AQ400 at 0.40 mg P/L, and one for the B+L at 0.04 mg P/L.

8. AUTOCLAVE MAINTENANCE

8.1. **After each digestion** – Clean the autoclave by wiping the inside of the chamber. Check for and remove any debris that may be left on the autoclave walls or on the trays. Record in the maintenance log.

8.2. **Weekly** – The Tuttnauer autoclave should be cleaned using ChamberBrite weekly. Place tablet/powder towards the back of the chamber and run a “glass

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cycle". Completely drain the reservoir and refill with DI water after the cycle. Run another glass cycle and repeat the draining and refilling of the reservoir.

8.3. **Monthly** – Clean and check the safety valve. Begin a glass cycle. Allow pressure to build to approximately 30 psi. Remove water reservoir cover. **CAUTION:** The next step will release hot steam. Pull the ring of the safety valve using a screwdriver or other tool. Hold the valve open for 2 seconds and release. Press the stop button to vent the cycle.

8.4. **Additional maintenance and logbook entries** – Record PMs, repairs, or phone troubleshooting performed by the manufacturer in the maintenance log. If the autoclave was used for purposes other than TP digestion, the description should also be recorded in the maintenance log.

8.5. **Consolidate Sterilizer Autoclave** –. This autoclave is only to be used as a backup for the Tuttnauer. It is not maintained by the Nutrients group.

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%20Health%20and%20Safety.aspx).

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

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

9.4. Neutralize the sulfuric acid digestion solution (4.2.1.) using sodium bicarbonate before disposal through the sink.

9.5. Residual concentrated acid must be neutralized using sodium bicarbonate before disposal in the sink.

9.6. Any sample waste, ammonium persulfate reagent waste, and diluent waste can be discarded through the sink with plenty of water.

10. 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.

10.1. EPA METHOD 365.1, Rev. 2.0 (1993).

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

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

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

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

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

10.7. DEP SOP NU-046, Procedure for TKN/TP/LLTP Glassware Cleaning.

10.8. DEP SOP NU-082, Analysis of Low-Level Total Phosphorus Using the Bran+Luebbe Segmented Flow Analyzer SOP.

10.9. DEP SOP NU-090, Determination of Total Phosphorus in Water using the AQ2 OR AQ400 Discrete Analyzer.

10.10. DEP SOP NU-091, Preparation of Waters for Total Kjeldahl Nitrogen Analysis (Copper Catalyst)

10.11. DEP SOP NU-092, Determination of Total Kjeldahl Nitrogen in Water using the Seal AQ2 Discrete Analyzer.

10.12. Quality Manual, Bureau of Laboratories, DEP.

10.13. Laboratory Health and Safety Plan.

Appendix of Significant Changes

02/25/2008 Updated SOP to comply with the latest revision of method: 365.1, *Revision 2.0*, August 1993.

02/10/2010 Changes were made in section 5 describing the use of an electronic data logger to collect temperature data from the autoclave.

02/03/2012 A description of the preparation of standard and spiking solutions was added. A new section was also added describing how to complete prep worksheets in LIMS and digestion sheets for each digestion procedure.

09/20/2012 The range for TP analysis on the Bran & Luebbe instrument was changed to 0.005-0.1 mg P/L. Two spike levels are now used per batch for LFBs and matrix spikes: 0.04 mg P/L and 0.4 mg P/L. The check standard was changed to 0.04 mg P/L for the TP analysis on the Bran & Luebbe instrument, and 0.4 mg P/L for TP analysis on the Seal AQ2 instrument.

02/06/2013 One spike level is prepared for the LFB and matrix spikes at 0.40 mg P/L per preparation batch. These are analyzed on the AQ2.

08/12/2014 Implement use of the electronic preparation worksheet (LIMS module). See section 5 for new instructions.

08/20/2015 Documentation of the digestion reagents in the LIMS prep worksheet was added to section 4.

12/13/2017 Documentation of the carrier solution in the LIMS prep worksheet was added to section 4. Preparation instructions for sample dilutions were added to section 5.

12/18/2018 Added a section for autoclave maintenance (section 8). Added discard details for reagents and samples to the waste management section (section 9). Added

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requirement to document bottle label checks in the digestion sheet (5.19.4). Section 4 and 5 were edited for clarity. Updated the reference for the Determination of TKN in soils using the AQ2 Discrete Analyzer.

12/12/2019 Updated sections 3, 5, and 8 to include instructions for use and maintenance of the 3870 ELB Tuttnauer Autoclave and Consolidated Sterilizer Autoclave.

11/24/2021 Added tables for instrumentation, equipment, supplies, chemicals, and standards. Updated autoclave maintenance and temperature recording procedures. Added table of contents.

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