DETERMINATION OF TOTAL KJELDAHL NITROGEN IN WATER USING THE SEAL AQ2 DISCRETE ANALYZER

TABLE OF CONTENTS:

Page

1. SCOPE AND APPLICATION 2
2. SUMMARY OF THE METHOD 2
3. EQUIPMENT AND SUPPLIES 2
4. REAGENTS AND STANDARDS 3
5. SAMPLE HANDLING AND PREPARATION 7
6. SAMPLE ANALYSIS AND QUANTITATION 7
7. DATA ARCHIVAL 10
8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES 11
9. MAINTENANCE/TROUBLESHOOTING 12
10. SAFETY/HAZARDOUS WASTE MANAGEMENT 13
11. REFERENCES 14
12. APPENDIX OF SIGNIFICANT CHANGES 15

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

1. SCOPE AND APPLICATION

1.1. The Seal AQ2 Discrete Analyzer is used for the determination of total Kjeldahl Nitrogen (TKN) in ground, surface, and saline waters, and domestic and industrial wastes. The calibration range is from the method detection limit (MDL) 0.080 to 4.0 mg N/L. The range may be extended by dilution up to a limit of 10 mg N/L of organic N in the sample prior to digestion. The practical quantitation limit (PQL) is 0.20 mg N/L.

1.2. The tests covered by this SOP are W-TKN and W-TKN-F.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method 351.2, Rev. 2.0 (1993) and Seal AQ2 method EPA-111-A Rev. 4.

2.2. Prior to analysis, digestion converts free ammonia and organic nitrogen compounds to ammonium sulfate (DEP SOP NU-091).

2.3. Ammonium reacts with salicylate and hypochlorite in a buffered, alkaline solution in the presence of sodium nitroferricyanide (pH = 12.4-12.7) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm.

2.4. Interferences: The working buffer eliminates precipitation of calcium and magnesium hydroxides. Samples that are turbid must be filtered prior to determination. High nitrate concentrations (>10 mg N/L) interfere with the TKN preparation procedure. The interference can be either negative or positive. For any samples with a known nitrate concentration of 10 mg N/L or higher the TKN result should be qualified with a J qualifier or the sample diluted sufficiently to decrease the nitrate concentration to below 10 mg N/L PRIOR to digestion.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation - Seal AQ2 Discrete Analyzer.

3.2. SEAL AQ2 consumables:
- 3.2.1. Reaction segments
- 3.2.2. Reagent containers
- 3.2.3. 1.2 mL sample cups

3.3. Glassware and pipettes – Assorted Class A type volumetric glassware, glass beakers, and variable Eppendorf pipettes.

3.4. Assorted clear and brown Nalgene bottles for reagent storage

3.5. Vacuum filtration apparatus with glass fiber prefilters

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.6. Magnetic stirring and heating apparatus and stir bar

3.7. 0.45 µm filters and syringes

3.8. Cleaning swaps or Q-tips

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

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water. Before preparing reagents or standards, or running samples, 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 preparations must be documented in the Reagent and Standard Logsheet. This excel sheet is located in the TKN-Cu folder in the REAGENT_PREP folder on the Nutrients drive and needs to be updated whenever a reagent is newly prepared. 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 or in the Reagent and Standard Logsheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased standard, reagent, and the standard reference material is maintained in the REAGENT_PREP folder on the Nutrients drive. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents and standards 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:

4.1.1. Ammonium Chloride, NH_4Cl

4.1.2. DI Water, ammonia-free

4.1.3. Glycine, $C_2H_5NO_2$

4.1.4. Sodium Salicylate, $NaC_7H_5O_3$, (Sigma-Aldrich, S3007-500G)

4.1.5. Sulfuric Acid, concentrated, H_2SO_4

4.1.6. Sodium Hydroxide, NaOH

4.1.7. Sodium Hypochlorite, NaOCl (approx. 6.0 % NaOCl, without additives)

4.1.8. Sodium Nitroferricyanide (Nitroprusside), $Na_2Fe(CN)_5NO*2H_2O$

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

4.1.9. Sodium Chloride, NaCl

4.1.10. Sodium Potassium Tartrate, $KOCO(CHOH)_2COONa*4H_2O$

4.1.11. Sodium Phosphate, Dibasic Heptahydrate, $Na_2HPO_4*7H_2O$

4.1.12. β-Glycerophosphoric Acid, Disodium Salt, Pentahydrate, $C_3H_7Na_2O_6P*5H_2O$

4.1.13. Potassium Dihydrogen Phosphate, KH_2PO_4

4.1.14. Standard Reference Material (ALTQC)

4.1.15. Ethylenediamine Tetraacetic Acid (EDTA) disodium salt hydrate

4.1.16. Hellmanex

4.2. Reagents:

4.2.1. **Stock Sodium Hydroxide, 20 % Solution** - In a 1 L volumetric flask, add 200 g NaOH (4.1.6.) to approx. 800 mL DI water while stirring slowly. Bring to volume with DI water. Do not filter. Holding time, 180 days.

Caution: Solution will become hot as sodium hydroxide is added to water.

4.2.2. **Stock Buffer** - In a 1 L volumetric flask, add 254.0 g sodium phosphate dibasic heptahydrate (4.1.11.) and 20 g NaOH (4.1.6.) to approx. 700 mL DI water. Stir until dissolved, approx. 60 minutes, then bring to volume with DI water. Store at room temperature (if the reagent is stored cold, precipitation can occur). Holding time, 60 days.

4.2.3. **Sodium Potassium Tartrate, 10 % Solution** - In a 2 L beaker, dissolve 200 g of sodium potassium tartrate (4.1.10.) in approx. 1.2 L of DI water. Add two to three pellets of NaOH (4.1.6.) and boil for 45 minutes to drive away any residual ammonia contamination. Cover, and after the solution has cooled down to room temperature, bring it to volume in a 2 L volumetric flask. Adjust the pH to that of DI water (approx. pH 6-7) by adding conc. H_2SO_4 (4.1.5.) dropwise, until the desired pH is reached. Store in refrigerator. Holding time, 180 days.

4.2.4. **Stock Color Reagent (sodium nitroferricyanide solution; 30 g/L)** - Dissolve 3 g sodium nitroferricyanide (4.1.8.) in a 100 mL volumetric flask and bring to volume with ammonia free DI water. Store the solution in a brown bottle in a refrigerator. Holding time, 30 days. Re-prepare if the solution develops a blue-green tint.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Caution: Can produce cyanide gas if in contact with acid. Refer to safety section for handling and disposal (10.7.).

4.2.5. **Stock Salicylate Solution**- Using a 1 L beaker and magnetic stirring apparatus, dissolve 150 g anhydrous sodium salicylate (4.1.4.) and 20 g sodium hydroxide (4.1.6.) in approx. 600 mL DI water. Transfer to a 1 L volumetric flask and bring to volume. Filter then store the solution in an opaque bottle in the refrigerator. Holding time, 60 days.

4.2.6. **Working Color Reagent (salicylate/nitroferricyanide solution)** - To 40 mL of the Stock Salicylate Solution (4.2.5.), add 0.25 mL of the Stock Color Solution (4.2.4.). This working reagent may be prepared/mixed directly in a 40 mL (wedge) AQ2 reagent reservoir. Holding time, 2 days.

4.2.7. **Working Buffer** - Use a clean and empty 100 mL volumetric flask and combine the reagents in the stated order. Add 50 mL of 10 % stock sodium potassium tartrate (4.2.3.) to 20 mL of stock buffer solution (4.2.2.) and mix. Add 18.4 mL of 20 % NaOH (4.2.1.) solution to the mixture. Dilute to the mark with DI water and mix well. Holding time, 3 days.

4.2.8. **Working Hypochlorite** - To a 50 mL volumetric flask containing approx. 30 mL DI water, add 10.0 mL hypochlorite (4.1.7.). Bring to volume. *Please check the hypochlorite concentration of the bleach used and adjust the volume used as necessary*. Holding time, 3 days.

Note: Stock Hypochlorite (4.1.7.) should be stored in the refrigerator.

4.2.7. **Alkaline EDTA Rinse (Cuvette Wash)-** In a 1 L flask, add 10 g of sodium EDTA (4.1.15.) and 20 g NaOH (4.1.6.) to approx. 600 mL of DI water. Dilute to volume when dissolved and mix well. Used for cuvette cleaning.

4.2.8. **Hellmanex Cleaning Solution-** Add 0.8 mL of Hellmanex (4.1.16.) to 40 mL of DI water. This solution may be prepared/mixed directly in a 40 mL (wedge) AQ2 reagent reservoir. Used for cuvette cleaning. Do not use if running phosphate analysis on same instrument.

4.3. Standard and spiking solutions:

4.3.1. **NH_3 stock (1000 mg N/L) for working standards** - To a 1 L volumetric flask containing about 500 mL DI water, add 3.819 g ammonium chloride (4.1.1.), which has been dried for 12-24 hours at 105 ^oC. Dilute to the mark and invert 12 times. Refrigerate. This solution is also used for ammonia analysis (DEP SOP NU-095, section 5.3.1.). Holding time, 180

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

days.

4.3.2. **TKN working calibration standard (CAL STD, 4 mg N/L)** - To a 1 L volumetric flask containing approximately 600 mL DI water add 2.0 mL conc. H_2SO_4 (4.1.5., final acid concentration 0.2 % (v/v)) followed by 4.0 mL NH_3 stock standard (1000 mg N/L, 4.3.1.) and fill up to the mark with DI water. Invert 12 times to mix. Store this standard at room temperature and digest according to DEP SOP NU-091. Holding time, 30 days.

4.3.3. **CCV (1 mg N/L)** - To a 1 L volumetric flask containing approximately 600 mL DI water add 2.0 mL conc. H_2SO_4 (4.1.5.) followed by 1.0 mL NH_3 stock standard (1000 mg N/L, 4.3.1.) and fill up to the mark with DI water. Invert 12 times to mix. Store this standard at room temperature and digest according to DEP SOP NU-091. Holding time, 30 days.

4.3.4. **ALTQC standard** - The quality control standard (4.1.14.) is prepared from the stock standard according to the manufacturer's instructions and digested according to DEP SOP NU-091. The supplier for the QC stock standards (e.g. Environmental Resource Associates) differs from the supplier of the calibration standards. The stock ALTQC standard may be diluted appropriately so that the concentrations falls within the calibration range. For the concentration and expiration date of the ampule see the manufacturer's certificate. Once prepared according to the manufacturer's instructions, the stock solution has a holding time of 90 days. If diluted further as a working solution, the holding time is 30 days.

4.3.5. **TKN/TP Organic Spiking Stock Solution (TKN: 150 mg N/L, TP: 50 mg P/L)** - Dry approx. 1.1 g glycine (4.1.3.) overnight at 60 °C. Add 0.8040 g glycine and 0.4941 g β-glycerophosphoric acid (4.1.12.) to a 1 L volumetric flask containing approx. 400 mL DI water. Bring to volume with DI water. Invert 12 times. Do not acidify. Store in refrigerator. Holding time, 180 days.
This solution is used to prepare laboratory fortified blanks (LFB), matrix spikes (MS) and matrix spike duplicates (MSD). This solution is also used for the analysis of TP and TKN in soils (DEP SOPS NU-084 and NU-081, respectively).

Note: **Glycine:** $C_2H_5NO_2$, Formula weight: 75.07 g/mol.
Molecular weight factor = 14.0067/75.07 = 0.1866.
β-glycerophosphoric acid: $C_3O_{11}H_{17}PNa_2$, Formula weight: 306.11 g/mol. Molecular weight factor = 30.9738/306.11 = 0.1012.

4.3.6. **TKN/TP PQL standard (PQCK2) (TKN: 0.20 mg N/L, TP: 0.06 mg P/L)** - To a 1 L volumetric flask, containing about 500 mL of DI water, add 200 µL of NH_3 stock standard (1000 mg N/L, 4.3.1), 60 µL of TP stock standard (1000 mg P/L, see DEP SOP NU-084, section 4.3.1.) and 2

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

mL of conc. H_2SO_4 (final acid concentration 0.2 % (v/v)). Bring to volume with DI water. Digest this standard according to DEP SOP NU-091. This solution is also used for the analysis of TP and TKN in soils (DEP SOP NU-084 and NU-081, respectively). Holding time, 30 days.

5. SAMPLE HANDLING AND PREPARATION

Refer to DEP SOP NU-091 for preparation, sample preservation, and handling details.

6. SAMPLE ANALYSIS AND QUANTITATION (SEE DEP SOP NU-033)

Note: • Digested solutions containing particulate matter should be filtered through a 0.45 μm filter prior to analysis on the AQ2 Discrete Analyzer and then redigested with dilution to confirm the result.
• Samples with organic N concentrations above 10 mg N/L must be diluted prior to digestion in order to report results without a J qualifier. (Subtract the ammonia concentration, if known, from the TKN result to calculate the organic N concentration). Add a comment on the run report to document this observation.
• Observe routine maintenance as recommended by the instrument manufacturer and enter any maintenance performed in the maintenance logbook.

6.1. Initial Preparation:

6.1.1. Remove reagents from the refrigerator. Check the expiration dates on all standards and reagents. Discard expired standards and/or reagents and re-prepare if necessary.

6.1.2. Select digested trays to be analyzed and vortex samples and standards thoroughly. Record worksheet ID/tray number on the Reagent and Standard Logsheet.

6.2. Calibration:

6.2.1. The instrument is calibrated using the digested 4.0 mg N/L working CAL STD (4.3.2).

6.2.2. The diluent for TKN is the digested blank (DEP SOP NU-091, 5.3.1.).

6.2.3. The auto calibration feature of the AQ2 is used in the analysis of TKN. The standards in the calibration are as follows (C2 to C6 are prepared by the instrument from the digested working calibration solution (4.3.2.)).

STD	Conc. (mg N/L)	Source
C1	0.00	Digested blank (6.2.2.)
C2	0.20	Instrument dilution of C7
C3	0.40	Instrument dilution of C7

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

C4	1.0	Instrument dilution of C7
C5	2.0	Instrument dilution of C7
C6	3.0	Instrument dilution of C7
C7	4.0	Digested CAL STD (4.3.2)

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled AQ2 reagent vessels with the following: Working Color (4.2.6.), Working Buffer (4.2.7.) and Working Hypochlorite (4.2.8.).

6.3.2. Reagent Location - The reagent tray, with plastic wedge-shaped containers is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. The buffer is in position 4, the color reagent in position 5, and the hypochlorite reagent is in position 6.

6.3.3. Controls - Fill one reagent vessel with the digested 1.0 mg N/L CCV (4.3.3.) and place it in position 10. Fill another vessel with digested blank (CCB, 6.2.2.) and place it in position 11.

6.4. Analysis Setup/Sequence setup:

6.4.1. Turn on the AQ2 Analyzer (large button on the right side at the back of the instrument) and the computer. To start the software double-click on the AQ2 Icon on the screen. The username is '**Instrument 1'** or **'Instrument 2'** and the password is 'password'.

6.4.2. Proceed to the daily maintenance (Section 9) if it is the first run of day. Otherwise continue to 6.4.3.

6.4.3. Check that the DI water reservoir is full and empty the waste container. Replace all used reaction segments.

6.4.4. In the software, go to the 'Setting' menu and select maintenance. Zero the reaction segment counter.

6.4.5. Setup of the analytical run - Click on the scheduling icon on the left side of the screen. Choose **Reagent Set 3**. Select a free tray and name the run using the format TKNYYYYMMDD001K or Q (K for instrument 1, Q for instrument 2). For the first run on instrument 1 on 03/24/2014 the name of the run would be TKN20140324001K. The sequence screen should appear. Starting at line 3, type the sample sequence in the 'Sample ID' column. Include at least 7 blank "C" cups at the end of the run to avoid a known timing issue affecting the final CCB/CCV results (see the Seal

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

AQ2 Method EPA-111-A, Rev. 4 for more information). Highlight the typed lines and select auto fill to fill the sequence numbers, and the **TKN** test. The test should appear in the requested test column to the right of the sample ID's. Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

A typical run sequence includes the following:

CCV (4.3.3.)
CCB (digested blank, 6.2.2.) – these checks are scheduled to bracket every ten cups on the run)
ERAQC# (ALTQC, 4.3.4.)
PQCK2 (4.3.6.)
MBLK
LFB
Spiked sample (MS) (4.3.5.) and **spike duplicate (MSD)/sample duplicate**
Samples
CCV
CCB
Only at the end of the analytical run: Seven blank "C" cups

Note: The AQ2 run sequence is set up to run batch QC before batched samples in order to screen for problems, as digested chemistries require quick decisions in the event of failing QC.

Note: Multiple trays can be run on the same sequence using one calibration. Indicate on the report which tray the BLK, CCV, and calibration standard 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.

6.4.6. Pouring the analytical run - Cup positions one and two are reserved for the calibration standards. The TKN calibration solution (6.2.1.) is placed in cup two. Cup 1 is left empty. Pour the samples according to the cup position on the bench sheet.

6.4.7. Double-click on the 'Run' icon on the left side of the screen. A dialog box will appear. Select the tray that contains the sequence to be analyzed. Click 'OK'. Another dialog box will appear. Check the auto-standardize box and click 'OK'. (If the analysis should use the previous calibration do not check the auto-standardize option.) Select 'OK' on the following dialog box.

6.4.8. The reagent levels are checked by the instrument and are displayed on the upper right corner of your screen. If the levels are sufficient for the entire run the analysis will start; if not, the run will stop and display the reagent

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

which has insufficient volume. Fill up the reagent and re-start the run according to 6.4.7.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for TKN in mg N/L.

6.5.2. 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 Section the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to section 4.3.2. The other standards used in the second order calibration are auto-diluted by the instrument from the high standard. The correlation coefficient and percent errors for the calibration standards are calculated by the instrument software.

6.6. Post-Run Procedure:

6.6.1. After the run has finished, print the AQ2 Test Parameters Report from the 'Settings' menu on the top of the screen.

6.6.2. Click on the 'Acceptance' menu located on the left side of the screen.

6.6.3. Click on the 'Accept All' Icon. This adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the naming convention described in 6.4.5. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current year's subfolder located in \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\TKN_COP\.

6.6.4. Click on the 'Reporting' icon on the left side of the screen. Check the reporting format 'TKN/TP' and print the run report. It contains the calibration and all the data. Select the chronological order report function to print your report.

6.6.5. Convert the excel file into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 1' or 'Nutrients: Seal AQ2 2'.

7. DATA ARCHIVAL

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

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

is labeled with the test (W-TKN, W-TKN-F), filename/s (TKNYYYYMMDD001K or Q), and initials of the analyst.

7.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, Reagent and Standard Logsheet, digestion temperature data logger report, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. The sample preparation worksheet is archived electronically in the Prep Worksheet Manager module in the LIMS system.

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. AQ2 instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in: \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\TKN_COP\.

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 \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

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

8.1. For method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to DEP SOPS NU-038 and NU-043.

8.2. A CCV (4.3.3.) and a CCB (digested blank, DEP SOP NU-091, 5.3.1.) are analyzed every 10 samples and at the beginning and end of each run. Recovery acceptance limits for the method blanks (MBLKs) and CCBs are < 0.080 and > -0.080 mg N/L. The check standard (CCV) recovery limits are 90 – 110 %

8.3. Each tray of digested samples contains a digested PQL level standard (PQCK2, 4.3.6.) and a certified standard reference material (ALTQC, 4.3.4.). Recovery acceptance limits are 70 -130 % for PQCK2 and 90 – 110 % for the ALTQC. The PQCK2 is also used for the ongoing MDL and PQL verification.

8.4. An MBLK, an LFB, a duplicate sample and/or duplicate matrix spikes are digested and analyzed with every batch of 10 or fewer samples (see DEP SOP NU-091). Acceptance limits for the LFB and matrix spike recoveries are 90 – 110 %. The RPD of sample/matrix spike duplicates must be less than 20 % for results above the PQL.

8.5. 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 second order calibration must be larger than

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

0.995. Acceptance limits for the percent error of the PQL level standard is ±30%. Acceptance limits for the percent error of calibration standards above the PQL are ±10%. 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.

9. MAINTENANCE/TROUBLESHOOTING

9.1. Empty and rinse the DI water reservoir at least three times. Refill to the top.

9.2. Empty the instrument rinse waste. Check that the instrument hazardous waste bottle isn't full.

9.3. In the SEAL AQ2 Program, go to 'Settings' then 'Maintenance.'

9.4. In 'Initialise,' press 'Zero Segments.' **Do not press 'OK' yet.**

9.4.1. Check to see if there are enough reaction segments from the day before to do maintenance. 2-3 unused is enough. Then press 'Cancel.' The probe will go to the first clean segment instead of Segment 1, spot 1.

9.4.2. If there are not enough clean segments, replace the segments. Double tap clean segments on the bench to knock out excess manufacturing particles, then place in the instrument. Press 'OK' to reset the used segments. The probe will go to Segment 1, spot 1.

9.5. Go to 'Diluter,' set the number of primes to 10, then press 'Start Primes.'

9.5.1. Watch the syringe piston to see if there are excess bubbles dragging from the top. A worn syringe piston can interfere with the calibration auto-dilution and analysis. Replace if necessary.

9.6. Clean the aspiration bath with a Q-tip or cleaning swap.

9.6.1. The particles from manufacturing can get caught in the cuvette. Daily cleaning of the aspiration bath helps prevent this from occurring.

9.7. In a reagent wedge in position 1, place either the Hellmanex Cleaning Solution (4.2.8.) or the Alkaline EDTA Rinse (4.2.7.). Go to 'Cuvette Functions', then press 'Extra Wash.'

9.7.1. Watch the glass sampler probe cleaner. A water vortex should be visible when the sampler probe is being washed and liquid should not drip out.

9.7.2. Contamination between samples and reagents can occur if the probe cleaner is not operating properly. Liquid drips out of the glass probe cleaner when the probe creates a wider hole at the bottom of the glass (will look oval). This can occur over time or if the sampler probe is bent. Replace the probe cleaner and sampler probe if necessary.

9.8. After 'Extra Wash' is done, set the number of 'Auto Wash' to 5. Then press 'Auto Wash.'

9.8.1. Make sure the aspiration bath is filling and emptying each time. Change

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

the two peristaltic tubing going to the aspiration bath if it is not.

9.9. After 'Auto Wash' is done, press 'Extra Wash' again.

9.10. After the second 'Extra Wash' is done, press the 'Auto Wash' again.

9.11. To adjust the aspiration, remove the cover from the top of the cuvette/lamp. Press 'Test Aspiration' and observe where the water line begins. It should be around 2 inches out of the cuvette. Repeat 'Test Aspiration' 2 more times, then check that the 4^{th}, 5^{th}, and 6^{th} repetition are in the same spot.

9.11.1. To make the water line come out of the cuvette further, increase the number of steps. Decrease the number of steps if it is too far out. Then repeat the above test.

9.11.2. If the water line is not around the same location in each repetition, random errors may occur in analysis. Replace the peristaltic tubing on the pump located next to the lamp.

9.12. In 'Initialise,' press 'Zero Segments,' and replace all used segments. Then press 'OK.'

9.13. Close the maintenance window then go to 'Settings' → 'Daily Startup' → 'Continue.' Write down the highest voltage and the corresponding filter in the maintenance book.

9.13.1. The highest voltage filter should remain the same for each instrument. A drastic change in voltage from day to day indicates a lamp going bad or an electrical issue.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

10.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

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

10.4. The solution used to digest samples contains copper, which is a hazardous waste and toxic. All waste from the instrument, digested samples, and standards made with digestion solution should be collected in bottles clearly labeled as hazardous waste. The waste should be disposed as acidic metals waste.

10.5. Once instrument waste containing copper is filled and removed from the instrument it can be stored for no longer than three working days within the hazardous waste storage of the analytical laboratory area.

10.6. Rinse waste from the instrument that can be disposed of in the sink is to be accompanied by tap water.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

10.7. The sodium nitroprusside (4.1.8.) used to prepare the color reagent produces cyanide gas if it encounters acid. Wear all personal PPE when preparing and thoroughly rinse flasks and containers after acid washing. Dispose excess sodium nitroprusside crystals by lab packing. Excess color reagent can be disposed of down the sink when flushed thoroughly with water.

11. REFERENCES

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.

11.1. EPA Method 351.2, Rev. 2.0 (1993).

11.2. Seal AQ2 Method EPA-111-A, Rev. 4, Total Kjeldahl Nitrogen-N (Copper Catalyst) in Drinking, Ground and Surface Waters, Domestic and Industrial Wastes.

11.3. DEP SOP NU-033, Summary of Analysis Procedure for the General Chemistry and Nutrients Workgroups.

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

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

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

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

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

11.9. DEP SOP NU-081, Determination of Total Kjeldahl Nitrogen in Soils using the Westco SmartChem Discrete Analyzer.

11.10. DEP SOP NU-084, Determination of Total Phosphorus in Soils using the AQ2 Discrete Analyzer.

11.11. DEP SOP NU-095, Determination of Ammonia in Water by Gas Diffusion Segmented Flow Analysis.

11.12. DEP SOP LB-006, Standard Operating Procedure for Records Maintenance and Storage.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

11.13. Quality Manual, Bureau of Laboratories, FDEP.

11.14. Laboratory Health and Safety Plan.

Appendix of Significant Changes

10/20/2009 Some minor corrections were made in section 4.

08/20/2010 Procedural clarifications some corrections mainly to section 4 and 6.

08/09/2011 Preparations of the PQL standard and spiking TKN solution were modified and now include phosphorous (these solutions also used for TKN and TP analysis in soils).

12/10/2019 Clarified Section 3. Added procedures for preparation of Alkaline EDTA Rinse and Hellmanex Cleaning Solution to Section 4. Section 6 was modified to clarify running multiple trays on one sequence. Added ongoing MDL/PQL verification information and acceptance limits for calibration standards recoveries to Section 8. Section 9 maintenance and troubleshooting was added. Section 10 safety and waste disposal procedure was clarified.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.