

Standard Operating Procedure (SOP) for
Standard Operating Procedure (SOP) For Total Nitrogen

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Table of Contents

1.0	Identification of test method	3
2.0	Applicable matrix or matrices.....	3
3.0	Method detection limit (MDL) / Limit of quantitation (LOQ)	3
4.0	Scope and application, including components to be analyzed.....	3
5.0	Summary of the test method	3
6.0	Definitions.....	3
7.0	Interferences.....	4
8.0	Safety	5
9.0	Equipment and supplies	5
10.0	Reagents and standards	6
11.0	Sample collection, preservation, shipment and storage	6
12.0	Quality control	7
13.0	Calibration and standardization	9
14.0	Procedure	10
15.0	Calculations.....	14
16.0	Method performance	16
17.0	Pollution prevention.....	16
18.0	Data assessment and acceptance criteria for quality control measures	17
19.0	Corrective actions for out of control data	18
20.0	Contingencies for handling out of control or unacceptable data.....	18
21.0	Waste management	18
22.0	References	18
23.0	Any tables, diagrams, flowcharts, and validation data.....	19
24.0	Additional notes.....	23
	SOP Signature Page	24

1.0 Identification of Test Method:

- 1.1 This SOP for VTDEC is based on Hach/Lachat method 10-107-04-1-C for the analysis of nitrate/nitrite as nitrogen in water, with reference to Standard Method SM 4500-N C Modified.

2.0 Applicable matrix or matrices:

- 2.1 This method determines the amount of total nitrogen in drinking, ground, and surface water and in saline, domestic and industrial wastewater, and soil extracts.

3.0 Method detection limit (MDL) / Limit of quantitation (LOQ):

- 3.1 Method Detection Limit (MDL) is approximately equal to the LOQ, and is used to calculate the Practical Quantitation Limit (PQL). This information is summarized in section 23.0
- 3.2 The PQL for is Total Nitrogen 0.10 mg/l.

4.0 Scope and application, including components to be analyzed:

- 4.1 This method determines nitrate/nitrite as nitrogen in water in liquid matrices by automated flow injection analysis.
- 4.2 The applicable range is 0.05 to 2.0 mg N/l.

5.0 Summary of test method:

- 5.1 A small sample volume is combined with an alkaline persulfate solution, and digested at 121 C / 15 psi. This process digests ammonia, nitrite and nitrogenous organic materials, oxidizing them to form nitrate.
- 5.2 Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520nm. Nitrite alone also can be determined by removing the cadmium column.

6.0 Definitions:

- 6.1 Calibration Blank (CB) - A volume of reagent (0.2 % sulfuric acid) 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.
- 6.3 Method Blank (MB) - A laboratory reagent (0.2 % sulfuric acid) blank that is treated exactly as a sample, and is used to monitor lab contamination. Digested sample and QC results are corrected for blank contamination using the averaged Method Blank results from an analytical run.
- 6.4 Filter Blank (FB) - A laboratory reagent (0.2 % sulfuric acid wash) 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.

- 6.5 Laboratory Duplicate (LD) - Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. The lab duplicates are treated exactly as the samples. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation or storage procedures.
- 6.6 Matrix Spike (MS) - A routine environmental sample with a known concentration of analyte added. The added analyte should have the same concentration as the Laboratory Control Sample (LCS), and be treated exactly as the samples.
- 6.7 Laboratory Control Sample (LCS) - A laboratory reagent blank with a known amount of analyte added. The added analyte should have the same concentration used for the Matrix Spike (MS), and be treated exactly as the samples.
- 6.8 Laboratory Control Sample – Low (LCS-Low) - A laboratory reagent blank with a known amount of analyte added. The added analyte should have a concentration at or near the PQL, and be treated exactly as the samples.
- 6.9 Initial Calibration Verification (ICV) - The ICV is obtained from a source other than the calibration standards, and treated exactly as the calibration standards.
- 6.10 Method Detection Limit (MDL) - The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.
- 6.11 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.
- 6.12 Limit of Quantitation (LOQ) – The LOQ is approximately equal to the PQL. A CCV Low is included in each analysis at 1-2 times the PQL.
- 6.13 Practical Quantitation Limit (PQL) - The laboratory's reporting limit, which is 2 to 5 times the minimum detection limit.
- 6.14 Continuing Calibration Verification Solution (CCV) - A calibration standard used to monitor instrument performance.
- 6.15 Continuing Calibration Blank (CCB) - A calibration blank standard used to monitor instrument performance.
- 6.16 Proficiency Evaluation (PE) – Certified solution of method analyte that is unknown to the analyst.
- 6.17 Data Quality Management (DQM) – Defines various quality control samples or sample sets within a tray protocol. These can be CCB/CCV Mid after a calibration curve and every ten samples, at the end of an analytical run; a Method Blank/LCS, a sample and its duplicate / MS.

7.0 Interferences:

- 7.1 Sulfide can rapidly and significantly reduce cadmium column efficiency.
- 7.2 Residual chlorine can oxidize the cadmium column.
- 7.3 High concentrations of iron, copper or other metals can give low results. EDTA is added to the buffer to reduce this interference.

- 7.4 Turbidity / sediment can interfere, and can be removed by filtration through a 0.45 μ m pore diameter membrane filter prior to analysis.
- 7.5 Oil and grease in high concentrations can coat the surface of the cadmium. Eliminate this by pre-extracting the sample with an organic solvent.

8.0 Safety:

- 8.1 Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) are stored online at www.thermofisher.com, and with the Lab Safety Officer (LSO).
- 8.2 The Laboratory Safety Plan is available for review as a hardcopy, and on the State of Vermont intranet (Y drive) and used by personnel involved in the analysis.
- 8.3 The following reagents are known to be toxic or hazardous. For more detailed explanations consult the MSDS/SDS information, and Lab Safety Plan.
 - 8.3.1 Sulfuric acid
 - 8.3.2 Ammonium hydroxide
 - 8.4.3 Sodium hydroxide
 - 8.4.4 Phosphoric acid
 - 8.4.5 Ammonium chloride
 - 8.4.6 Cadmium
 - 8.4.7 Sulfanilamide
 - 8.4.8 Potassium persulfate

9.0 Equipment and Supplies:

- 9.1 Balance(s) - Analytical, capable of accurately weighing to the nearest 0.001g
- 9.2 Class 'A' volumetric flasks and lab ware as needed.
- 9.3 Rainin EDP pipette w/ disposable tips.
- 9.4 Flow injection analysis instrumentation designed to deliver and react sample and reagents in required order and proportion.
 - 9.4.1 Auto sampler.
 - 9.4.2 Auto dilutor.
 - 9.4.3 Multi-channel peristaltic pump with at least 4 channels.
 - 9.4.4 Reaction unit or mixing manifold.
 - 9.4.5 Colorimetric detector, including 80 μ l flow cell with 10mm path length.
 - 9.4.6 Interference filter 480nm \pm 10nm band pass.
 - 9.4.7 Cadmium reduction column
 - 9.4.8 Data handling system
- 9.5 50ml polyethylene screw-top centrifuge tubes as needed
- 9.6 16 x 125 mm glass screw top tubes with marking spot, threaded polypropylene (liner less) caps.
- 9.7 16 x 100 mm glass test tubes as needed
- 9.8 Filters as needed.

10.0 Reagents and standards:

- 10.1 Carrier/Diluent 0.2 % Sulfuric acid. In a 2L volumetric flask with 1800 ml D.I. water, add 4ml low nitrogen sulfuric acid (Fluka #84727) and dilute to volume. Prepare daily.
- 10.2 Digestion Reagent-Each sample needs 5ml digestion reagent. Prepare volume according to Table A.

Volume	Potassium Persulfate ($K_2S_2O_8$)	Sodium Hydroxide (NaOH)
1 L	20.0 g	6.0 g
500 ml	10.0 g	3.0 g
250 ml	5.0 g	1.5 g
100 ml	2.0 g	0.6 g

- 10.3 Ammonium Chloride Buffer-In a 2 L volumetric flask dissolve 170.0g ammonium chloride (NH_4Cl) and 2.0g disodium EDTA ($Na_2-EDTA \cdot 2H_2O$) in approximately 1800ml D.I. water. Add 16.0ml ammonium hydroxide (NH_4OH). Dilute to 1L with D.I. water and mix on magnetic stir plate until dissolved. Adjust pH to 8.50 ± 0.05 with ammonium hydroxide. Hold time is one month.
- 10.4 Sulfanilamide Color Reagent-To a 1L volumetric flask add 40.0g sulfanilamide, and enough D.I. water to wet. Then add 100ml of 85% phosphoric acid (H_3PO_4) and swirl to mix, add 500 ml D.I. water and mix until dissolved. Add 1.0g N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Dilute to volume and mix on magnetic stir plate until dissolved. Store in a dark bottle. Hold time is 1 month.
- 10.5 Primary standard, Nitrate as N 1000mg/l (Spex #AS-NH3N9-2Y). Expiration date is given by the manufacturer or one year from date opened.
- 10.6 Secondary standard, Nitrate as N 1000 mg/l (ERA #052), Expiration date is one year from ship date.
- 10.7 Reduction efficiency standard, Nitrite as N 1000 mg/l, either of the following can be used: Spex #AS-NO2N9-2Y or ERA #053.

11.0 Sample collection, preservation, shipment and storage:

- 11.1 Samples are collected in pre-cleaned, disposable 50ml polyethylene screw top centrifuge tubes.
- 11.2 Samples are delivered to the lab by field personnel or courier.
- 11.3 Samples are acidified w/ low nitrogen sulfuric acid and stored at ≤ 6 C.
- 11.4 Sample hold time is 28 days.

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 laboratory capability, determination of minimum detection limit, periodic analysis of laboratory reagent blanks (method blanks), laboratory control samples, sample (matrix) spikes and duplicates, initial calibration verification solutions as checks on analytical performance and meeting a minimum correlation coefficient for the calibration curve. The laboratory maintains and monitors the data in the Lab Information Management System (LIMS). Definitions below may refer to a Data Quality Management (DQM) set, defined as one or more QC samples analyzed concurrently to monitor instrument performance.
- 12.2 Method Detection Limit (MDL) - MDLs are determined/verified annually, when a new operator begins work, or whenever there is a significant change in the method procedure. The MDL is determined by analyzing 7 samples at 0.1 mg/L, yielding a PQL of 0.1 mg/L.
- 12.3 Linear Dynamic Range (LDR) - The LDR must be determined initially or whenever a significant change in instrument response is observed or expected.
- 12.4 Initial demonstration of Ability (IDA) – An IDA is required by new analysts before reporting results. The reference method and lab SOP must be reviewed. The new analyst must observe the current analyst running at least one analytical batch, then analyze an analytical batch under current analysts supervision, and finally analyze and meet QC criteria for four replicates of LCS or ICV.
- 12.5 Proficiency Evaluation (PE) – Successful analysis of a PE sample at a minimum of one concentration must be completed prior to reporting results. Results should be within the test limits established by USEPA for the category of sample being tested.
- 12.6 Lab Reagent Blank aka Method Blank (MB) – are analyzed to determine if reagents contribute contamination to the process. An MB is paired with an LCS and referred to as a DQM set. This DQM set is analyzed with each analytical batch of 20 or fewer samples. Results should be less than ½ the PQL. If the result is greater than ½ the PQL, sample results in the corresponding analytical batch that are between the PQL and 2x the value of the Method Blank are given a remark code 'BH' (result may be Biased High).
- 12.7 Laboratory Control Samples (LCS) – A laboratory reagent blank with a known amount of analyte added. The added analyte should have the same concentration used for the Matrix Spike (MS), and be treated exactly as the samples. An LCS is paired with an MB and referred to as a DQM set. This DQM set is analyzed with each analytical batch of 20 or fewer samples. LCS recovery of 90 – 110 % is expected. Minimum frequency is 5%, or 1 LCS per analytical batch, whichever is greater.

- 12.8 Laboratory Control Sample - Low (LCS Low) – A laboratory reagent blank with a known amount of analyte added at or near the PQL. The added analyte should be treated exactly as the samples. An LCS Low is run once during the analysis with an expected recovery of 75 – 125 %.
- 12.9 Matrix Spike (MS) - A routine environmental sample with a known concentration of analyte added, and is paired with an unspiked sample. The added analyte should have the same concentration as the Laboratory Control Sample (LCS), and be treated exactly as unspiked samples. Recovery of 85 – 115% is expected. Minimum frequency is 5%, or 1 LCS per analytical batch, whichever is greater.
- 12.10 Lab Duplicate - Two portions of the same environmental sample treated identically throughout the analytical procedure, and treated exactly as the samples. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation or storage procedures.
- 12.11 Initial Calibration Verification (ICV) - The ICV is obtained from a source other than the calibration standards, and treated exactly as the calibration standards. The frequency is once per analysis, immediately after the calibration is acquired. Expected recovery is within 10 % of the true value. Re-prepare and rerun if the ICV recovery is outside expected limits.
- 12.12 Continuing Calibration Verification (CCV) – are calibration standards used to monitor instrument precision and performance. Data is kept in LIMS and monitored by the QC Officer.
 - 12.12.1 CCB - Continuing Calibration Blank is analyzed after the calibration and every ten samples thereafter. It is paired with the CCV Mid and referred to as a Data Quality Management (DQM) set. CCB results should be less than $\frac{1}{2}$ the PQL. If the CCB fails, the analysis must be stopped, the cause determined and the instrument recalibrated. The CCB data must be kept on file with the sample analysis data.
 - 12.12.1 CCV Low - is a low level calibration standard near or at the PQL, and is analyzed once during the run after the calibration. The expected recovery for the CCV Low is within 70 -130% of the true value. If the CCV Low fails, re-run once. If still unacceptable, the analysis must be stopped, the cause determined and the instrument recalibrated. The CCV Low data must be kept on file with the sample analysis data.
 - 12.12.2 CCV Mid - is a midlevel calibration standard analyzed after the calibration is complete and every ten samples thereafter. It is paired with the CCB and referred to as a DQM set. The expected recovery for the CCV Mid is $\pm 10\%$ of its true value. If the CCV Mid fails, the analysis must be stopped, the cause determined and the instrument recalibrated. The CCV Mid data must be kept on file with the sample analysis data.

- 12.13 Correlation Coefficient – The minimum correlation coefficient for Chloride is 0.995 or greater.
- 12.14 % Residual for Calibration Standards – The expected % residual for duplicate calibration standards is $\leq 10\%$.

13.0 Calibration and standardization:

- 13.1 Intermediate standard –Nitrate as N 10 mgN/l. Dilute 1.0 ml of 1000 mg/l primary stock to 100 ml with DI water.
- 13.2 Working standards - Use intermediate nitrate standard to prepare 100 ml volumes of working standards as noted in table below. Bring to volume with D.I. water then acidify with 200 μ l low nitrogen sulfuric acid. Dispense into 50 ml plastic screw top centrifuge tubes. Hold time is 28 days.

Volume (ml) of 10 mg/l Nitrate Stock	Concentration mg/l
20	2.0
10	1.0
5	0.5
1	0.10
0.5	0.05
0.0 (100 ml D.I. water)	0.0

- 13.3 Prepare (NO₃-N) Nitrate as N ICV at a mid-calibration level using other than primary stock solution. Bring to 100 ml volume with D.I. water then acidify 200 μ l low nitrogen sulfuric acid. Dispense into 50 ml plastic screw top centrifuge tube. Hold time is 28 days.
- 13.4 Prepare (NO₂-N) Nitrite as N reduction efficiency standard. Dispense 100 μ l nitrite stock 1000 mg/l to 100 ml volumetric flask. Dilute to volume. Do not acidify.
- 13.5 Prepare (NO₃-N) lab control sample (LCS and MS) standard, 100 mg/l NO₃. Use Rainin pipet to make a 1:10 dilution of the primary stock solution into a test tube. The lab uses 100 μ l of 100mg/l stock + 10 ml volume to yield a concentration of 1 mg/l.
- 13.6 Prepare TN lab control sample (LCS and MS) stock standard, 100 mgN/l. Dissolve 0.2627 mg of L-Glutamic acid in 250 ml volumetric flask with D.I. water. The lab uses 25 μ l of 100mg/l stock + 5 ml volume to yield a concentration of 0.5 mg/l TN.
- 13.7 Prepare TN LCS Low stock. Dilute an aliquot of ERA Simple Nutrient QC (Item #505) to create an intermediate spiking solution. The final concentration of a blank spiked with this solution should be at or near the PQL of 0.1 mg/l.

- 13.8 Record the following information on the TN QC spreadsheet: Standards and QC prep, instrument model, Standard Method reference, test, analyst, test date, color reagent lot number and expiration date, primary and secondary standards with their respective concentrations, lot numbers, expiration dates. Information is updated with each date of analysis and saved in a folder on the instrument computer by clicking on the 'Save' radio button.

14.0 Procedure:

- 14.1 Sample Digestion-Prepare digestion reagent according to Section 10.2, Table A.
- 14.1.1 Remove samples from the refrigerator and bring them to room temperature. Check each sample to ensure that the pH is ≤ 2 .
Note: If sample pH is greater than 2 and the sample is within 24 hours of receipt then add 0.1 ml per 50 ml of sample. If after 24 hours, acidify and analyze sample including the proper remark code.
- 14.1.2 Write sample number on glass test tubes with a permanent marker. There are 2 method blanks, an LCS, and an LCS Low at the beginning of the digestion run. A digestion run can contain many batches (a batch consists of 20 samples) with each batch containing a method blank and an LCS. End the digestion run with 2 method blanks.
- 14.1.3 Add the following to each test tube:
Method Blank. Use 5 ml of acid wash for method blanks.
LCS. Add 25 μ l of the 100 mgN/l spike standard to 5 ml of the 0.2% acid wash to the tubes marked LCS.
LCS Low. Dispense a small volume of a second source QC standard to 5 ml of 0.2% acid wash to achieve a concentration at or near the PQL. Include 1 LCS Low per digestion batch.
Matrix Spike. Dispense 25 μ l of the 100 mgN/l spike standard to empty marked tube, then add 5 ml of well-mixed sample.
- 14.1.4 Use 'dilution' function on 10 ml Rainin pipet to draw / mix 5 ml digestion reagent and 5 ml of 'WELL shaken' sample. Dispense this mixed volume into a labelled glass tube and **cap immediately** and firmly. Ammonia can be lost if sample is not capped immediately upon addition of digestion reagent. Include a minimum of 5% spikes and duplicates.
- 14.1.5 Place racks in the autoclave and digest for 30 minutes at 121°C and 15 psi. Record digestion in autoclave log book. After cycle is complete, remove samples from autoclave, and let the samples cool to room temperature before analysis.
- 14.2 Prepare standards (Section 10.0) and reagents for analysis (Section 13.0). Install manifold, sample loop and filter. Turn on power to instrument via power strip. Turn on computer and log onto instrument. Double click on the Omnion icon.

- 14.3 Click on Run, Open and open Data folder. Select Total Nitrogen method and choose TN template.omn.
- 14.4 0.2 % sulfuric acid (Section 10.1) is used as carrier in 1 liter Erlenmeyer flask. Place auto dilutor tubing into this container. Begin pumping carrier and reagents through manifold. Tubing should be clearly labeled. Waste stream is saved and disposed of according to the Laboratory hazardous waste plan.
- 14.5 Initialize the auto sampler by clicking on *Configuration* and *Auto samplers*. Choose *Autosampler 1* tab and click *Initialize Autosampler*. After this is done, click on *Prime Auto dilutor*. Close dialog box.
- 14.6 Calibration standards. Place each prepared standard in its respective standard rack slot. Each standard is run in duplicate.
 - 14.6.1 The method timing and acquisition parameters should already be entered and optimized in the tray template. The calibration standards and QC elements and reduction efficiency standard should have been entered with their assigned values, acceptance limits and failure actions. See Lachat method #10-107-04-1-C for guidance.
- 14.7 Enter sample ID numbers via bar code scanner into the empty slots in the tray template. Place samples and QC into corresponding tray locations. Press 'Enter' to save entry and advance cursor.
 - 14.7.1 For samples diluted prior to digestion, check box in MDF column and enter dilution factor. It's helpful to include the dilution factor with the sample ID in the tray template.

As sample ID numbers are being scanned in, use the QC menu sheet on the bench to scan MB/LCS/MS into the tray template. Sample batches of ≤ 20 samples are bracketed by a method blank (MB), and lab control sample (LCS). Highlight and delete the remaining empty tray slots.
- 14.8 Enter DQM sets within the tray template. Move cursor into the gray *Sample No* column to the left of the *Cup No* column. Left click and drag cursor so that the rows with the CCB and CCV Mid are highlighted. Right click and select *Define DQM Set*. In the 'User Defined DQM Set' box under 'Scheduling Options' select 'After every N samples'. The DQM set is run after every 10 samples, (enter 10), and uncheck 'Close end of run' box. Click OK.
- 14.9 Press the 'Start' icon to begin the run.
- 14.10 Once analysis is complete, export data. Insert a thumb drive into the USB port. In top right corner of screen, go to *Run Properties* window and click on the *Run* tab. Click on *Export Data to file* to copy run data to thumb drive. Use cursor to highlight the Calibration curve peaks on the screen.

See section 14.3 for parsing instructions.
See section 14.4 for creating a QC batch.
See section 14.5 for data importing instructions.

- 14.11 Format and print the run report. Go to the tool bar and click on *Tools* and *Custom Report*. In tool bar, click on *Report*, and *Open Format*. Choose TN report format. Click to open the 8th icon (yellow) from the left. There are five tabs used to format the report.
- 14.11.1 Click on the *Sample* tab. Uncheck the *calibration standards* boxes. Click *Apply* in lower right of window.
 - 14.11.2 Click on *Table* tab. Check the *cup number* box. If the run included any instrument duplicates, auto or manual dilutions, check the boxes that pertain. Click *Apply* in lower right of window.
 - 14.11.3 Click on *Layout* tab. Check header information, change date of analysis. Click *Apply* in lower right of window.
 - 14.11.4 Click on *Calculations* tab. Under *Sample Preparation* check the boxes that apply so that results are multiplied by the auto or manual dilution factor. Click *Apply* in lower right of window.
 - 14.11.5 Click on the *Charts* tab. In the *Options* section, check *Calibration* and *Channel Data Display* boxes. In the *Channel Data Display* section, under *Select Channel(s) for Report*, #1 should be checked. Under *Display Options*, click on *Show ___ Peaks per Chart for All Peaks*. Enter 10. Click *Apply* then *Close* in lower right of window.
 - 14.11.6 Print analytical run report. On top of the screen, Click on *Print* icon, 7th from the left. Select *Print*. Save report format. Go to tool bar, click on *Report*, and *Save Format*. Choose TN, and OK. Close window. Print a copy of the *Standards/QC* spreadsheet to include with the analytical run report.
 - 14.11.7 Save an electronic copy of the *Standards/QC* spreadsheet to the folder by clicking on the '*Save*' radio button located on the spreadsheet. Save the desktop copy by clicking the '*Save*' icon at top left of screen.
- 14.12 Calculate TN results on instrument computer
- 14.12.1 Click on Icon '*Calculate TN results*' on lower toolbar to open macro. Enable macro, click on '*Calculate*' radio button.
 - 14.12.2 Navigate to thumb drive and open TN file to be calculated. Calculating will commence.
 - 14.12.3 Type in header info, and any sample dilution factors from the tray template. Check all formulas and cell references for accuracy.
 - 14.12.4 Set '*Print Area*' and print. Close this window without saving and remove thumb drive.

14.13 Create a QC Batch on LIMS networked computer

- 14.13.1 Log in to Sample Master. Click on the '*Data Entry*' icon in the Main Menu window. Highlight the '*Create QC batch*' then click on the '*Select*' option.
- 14.13.2 In the '*Matrix*' drop down box select '*Water*'.
- 14.13.3 In the '*Test*' drop down box select '*Nitrogen-Total Persulfate*'. Make sure that the '*Unassigned Samples*' box is checked. Select '*Retrieve*'.
- 14.13.4 In the '*QC Batch*' window, check the boxes of the sample numbers to be included in the QC batch, by referencing the TN (Calculated) Data sheet.
- 14.13.5 Click the '*New*' button to assign a QC batch ID to the checked samples. If the date of analysis is different from the date of the QC Batch ID, change it to reflect the date of analysis. Write this QC batch number on the front page of the TN data sheet.
- 14.13.6 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.
- 14.13.7 In the '*QC Batch*' window, use the drop down boxes to assign order and sample ID to the 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*'. Minimize Sample Master.

14.14 Parse the data on LIMS networked computer

- 14.14.1 Insert the thumb drive into a USB port on LIMS networked computer.
- 14.14.2 Open Windows Explorer. Open the following. Y drive, LIMS folder, Instrument Parsers Folder, Lachat Omnion 3 folder, TN Parser.xls.
- 14.14.3 Enter QC Batch, analyst initials, analysis date and OK. At the prompt, find and choose the file to be parsed. Click OK to commence parsing. Data will be displayed in Excel. Review results and QC numbers.
- 14.14.4 Delete any spaces or typed characters associated with sample ID's.
- 14.14.5 Data outside of calibration range cannot be reported. This should be deleted by row so as not to import it into the LIMS.
- 14.14.6 'Save' this spreadsheet, and close. Data will be saved in the '*Data2import*' folder.

14.15 Import Data into LIMS

- 14.15.1 Maximize Sample Master. Click on the Electronic Data Transfer (Green arrow) icon, second from the bottom. Highlight the phrase *Import All Files in One Directory* and click *Select*.
- 14.15.2 In the dropdown box choose TN 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 second icon from top, choose Result Entry, and select. Review the imported data.
- 14.15.3 Forward imported data to another analyst for review and validation.

14.16 Data Validation - Data is peer reviewed for errors or omissions by a chemist familiar with the method.

- 14.16.1 The calibration curve correlation, MS, LCS, CCV, ICV recoveries and RPD values should all be within control limits. The interval of the DQM set(s) is checked.
- 14.16.2 Sample Master is opened and the QC batch is retrieved by clicking on the second icon from the top. Select *Result Entry*. Select the desired QC batch from the QC batch drop down box.
- 14.16.3 Select the *Data to be validated* radio button. Data in Sample Master and the analytical report should agree. All relevant data should be included and should be within acceptable limits. Any out of control values should be properly flagged and/or commented.
- 14.16.4 Values to be validated should have their respective boxes checked. Once this has been done, click *Validate* then *Close*.
- 14.16.5 The analyst will initial and note the date that the data was validated on the front page of the analytical run report. The report is filed.

15.0 Calculations:

- 15.1 Total Nitrogen sample results are calculated using a macro on the instrument computer (Section 14.12). To calculate manually, subtract the average method blank value from the obtained result and multiply by 2. This accounts for the known contamination added to each sample by the digestion reagent, as well as the dilution of the sample.

$$[\text{Raw Result} - \text{Avg. MB}] \times 2$$

- 15.1 Calculate the MS percent recovery using the following equation:

$$[(C_s - C_u) / C] * 100$$

C = spike (stock) concentration (mg/l)
C_u = unspiked concentration (mg/l)
C_s = spiked concentration (mg/l)

If the recovery of the analyte falls outside the designated MS recovery range and the laboratory performance for that analyte is shown to be in control, the recovery problem with the MS is judged to be either matrix or solution related, not system related.

- 15.2 Calculate Relative Percent Difference (RPD). Analytical duplicate results are used to calculate a Relative Percent Difference (RPD). If the determined RPD values are not within established control limits, the source of the problem is identified and corrected before results can be submitted.

$$\frac{|(D_1 - D_2)|}{(D_1 + D_2)/2} * 100 = \text{RPD}$$

D₁ = larger duplicate value D₂ = smaller duplicate value

- 15.3 Laboratory Control Sample (LCS) – The Laboratory analyzes at least one LCS with each batch of 20 samples. Accuracy is calculated as percent recovery.
15.4 Laboratory Control Sample - Low (LCS Low) – The Laboratory analyzes at least one LCS Low with each digested batch of samples. Accuracy is calculated as percent recovery.

16.0 Method performance:

- 16.1 Continuing Calibration Verification (Mid-level) - For all determinations the laboratory must analyze a mid-range continuing calibration verification standard (CCV Mid) and a continuing calibration blank (CCB) following daily calibration, after every tenth sample, and at the end of the analytical run. The result of the CCV Mid must be within 10% of its true value. If the calibration cannot be verified within the specified limits, reanalyze the CCV Mid solution. If the second analysis of the CCV Mid confirms the calibration to be outside control limits, sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable CCV Mid result must be reanalyzed. The results of the calibration blank and CCV Mid solution must be kept on file with sample analyses data.

- 16.2 Continuing Calibration Verification (Low level) – the CCV Low is a low range calibration standard with a value 1 to 2 times the Practical Quantitation Limit (PQL). The PQL is approximately equal to the Limit of Quantitation (LOQ). A CCV Low must be run once during the analytical run, and its recovery must be within 30% of its true value.
 - 16.3 Initial Calibration Verification (ICV) - A mid-range ICV is prepared from a source other than the calibration standards, and run after the calibration standards and before any samples are analyzed. The value must be within 10% of the true value or the run is stopped, and the cause determined before analyzing any samples.
 - 16.4 Matrix Spike (MS) - The analyst must add a known amount of analyte to a minimum of 5 % of routine samples. In each case the MS aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration and source should be the same as that used in the laboratory-control sample. If the recovery of the analyte falls outside the control limits of 85-115%, the MS is judged out of control, and the source of the problem is identified and resolved before continuing analysis.
- 17.0 Pollution prevention:**
- 17.1 Laboratory policy is to purchase reagents and standards on an as needed basis. Reagents and standards are logged into the chemical inventory database with an associated expiration date, eliminating the need for a stockroom. The database is reviewed on an ongoing basis for expired reagents, which are disposed of through UVM Environmental Safety Facility.

18.0 Data assessment and acceptance criteria for quality control measures

Summary Table of Quality Control Procedures, Limits and Corrective Actions

Assessment	QC Procedure	Frequency	Limits	Corrective Action
Linearity of Calibration Curve	Correlation coefficient	Each calibration	≥ 0.995	Recalibrate
Indication of instrument precision.	Duplicate measurements	All standards	$< 10\%$ residual	Re-analyze. If still out of control, find source of problem then re-calibrate.
Verification of calibration curve against second source standard	ICV Mid	After each calibration	90 – 110%	Prepare again and recalibrate
Stability of the low end of the calibration	CCB	After ICV , every 10 samples and at end of run	$< \frac{1}{2}$ the PQL	Identify and correct problem
Stability of Calibration Curve	CCV Mid	After ICV , every 10 samples and at end of run	90 – 110%	Rerun CCV. If still out, recalibrate and rerun all samples not bracketed by an acceptable CCV
Stability of Calibration Curve	CCV Low	Once during the run	90 – 110%	Identify and correct the problem. Note that the CCV Low is 1-2 times the PQL which is \cong LOQ
Determine if methodology is In control	LCS	1 per batch	85 – 115%	Identify problem and correct, or prepare again and rerun sample
Indication of the effect of the sample matrix on the accuracy of the results	MS	5 % of all samples or 1 per batch, whichever is greater	85 – 115%	Prepare again and rerun
Indication of the effect of the sample matrix on the precision of the results	LD	5 % of all samples or 1 per batch, whichever is greater	10% RPD	Prepare again and rerun
Efficiency of column	Reduction/Efficiency standard	At the beginning and end of analytical run	$\pm 10\%$	Re-analyze. If still out of control replace cadmium column
Reagent water contamination	MB	1 per batch	$< \frac{1}{2}$ the PQL	Identify problem and correct.

Note: If sample values are reported from an analysis where any of the above limit criteria are exceeded, an appropriate remark code or sample note should be entered to justify reporting the results.

19.0 Corrective actions for out of control data:

19.1 See section 18.0

20.0 Contingencies for handling out of control data:

20.1 If a quality control measure is found to be out-of-control, and the data is to be reported, all samples associated with the failed quality control measure are reported with the appropriate data qualifying remark code found in Section 5.0 of laboratory QA Plan. In addition, final reports may include Order Comment written by analyst and/or supervisor, further qualifying data.

Sample Remark Codes *

Remark Code	Description
B	Reported value is associated with a lab blank contamination.
BH	Reported value may be biased high.
BL	Reported value may be biased low.
E	Estimated Value
D	Dilution resulted in instrument concentration below PQL.
H	Hold time exceeded.
I	Matrix Interference
O	Outside calibration range, estimated value.
OL	Outside Limit
P	Preservation of sample inappropriate, value may be in error.
S	Surrogate recovery outside acceptance limits.
T	Time not provided
W	Sample warm on arrival, no evidence cooling has begun.

21.0 Waste Management:

21.1 Waste generated from this analysis is disposed through ESF. A "waste tag" is filled out by the employee generating waste, which is then entered online for pickup. See Appendix II of Department of Environmental Conservation Laboratory, Final Laboratory Waste Management Plan.

22.0 References:

22.1 Standard Methods for Examination of Water and Wastewater, AWWA, APHA, 21th Ed. SM 4500-N C Modified.

23.0 Tables, diagrams, flowcharts and validation data:

23.1 Instrument Parameters

Under **Analyte** tab, Channel 1

Property	Value
Description	(none)
Channel OFF	(box not checked)
Method	FIA

Under **Analyte** tab, Channel 1,

Property	Value
Analyte Name	Nitrate- Nitrite Nitrogen
Concentration Units	mg/l
Calibration Fit Type	First Order
Clear Calibration	Yes
Force Through Zero	No
Calibration Weighting	None
Auto Dilution Trigger	Yes
% of High Standard	100
QuickChem Method	10-107-04-1-C
Chemistry:	Direct/Bipolar
Calibration by Height	No

Under **Timing** tab, Run

Property	Value
Method Cycle Period(s)	45
Sample Period (s)	15
Min. Probe in Wash Period	5
Pump standby active	No
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)	10
Inject period (s)	35
Time to valve (s)	24
Use retention time	No

Under **Timing** tab, Run, Channel 1, Nitrate-Nitrite Nitrogen

Property	Value
Expected inject to peak start (s)	26
Expected peak base width (s)	40

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

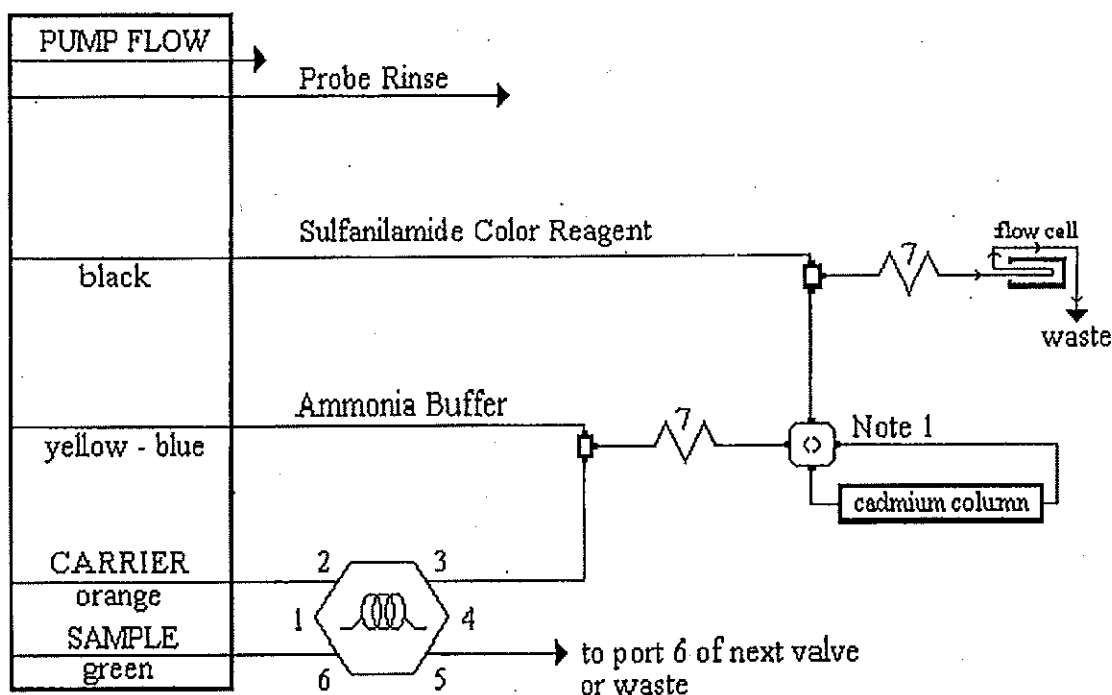
For Channel Independent Data, the Selected Items are (in order shown)

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

Nitrate Manifold Diagram

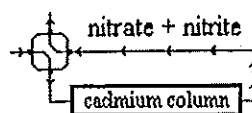
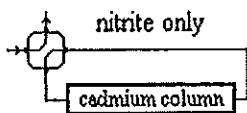


Carrier: Helium Degassed DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 $\mu\text{L}/\text{cm}$.
AE Sample Loop: 17 cm x 0.8 mm i.d.
QC8000 Sample Loop: 22.5 cm x 0.8 mm i.d.
Interference Filter: 520 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

7: 135 cm of tubing on a 7 cm coil support

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold



24.0 Additional notes:

Troubleshooting

- 24.1 Trouble is often traced to either mechanical or chemical sources. The following are suggestions to help prevent problems. For more detailed troubleshooting guidance, consult the Lachat training manual or call Lachat tech support at 800-247-7613
- 24.2 Mechanical problems can show up as air spikes, misshapen peaks or an unstable or noisy baseline. To minimize these potential problems, prior to beginning a test, do the following.
- 24.3 Reagents should be at room temperature prior to the run. Sparging reagents with helium is not needed.
- 24.4 Make sure all mixing manifold tubing connections are snug, but not tight enough to impede reagent flow.
- 24.5 While reagents are pumping through the manifold make sure there are no air bubbles in the flow cell. Remove the cell from the colorimeter and tap gently with finger to dislodge any bubbles.
- 24.6 Confirm that timing parameters are correct.
- 24.7 Chemical problems can show up as poorly shaped peaks, lower than expected peak areas or as an elevated baseline. Properly made and stored reagents can minimize these potential problems, and using reagents before the recommended expiration date.
- 24.8 Rainin EDP pipette is used to make standards, QC check samples and sample spikes. This pipette and attachments are serviced and calibrated annually. Calibration is verified quarterly.
- 24.9 Septic samples may have hydrogen sulfide present as well as high NH_3 and low NO_3 . If a strong rotten egg odor is noticed, dilute with acid wash 1:10. Hydrogen Sulfide will rapidly degrade column efficiency.
- 24.10 Nitrate/Nitrite samples without previous data or site history should be screened using EM QUANT Nitrate test strips (VWR Scientific 1-800-932-5000) and diluted prior to analysis.
- 24.11 ACS grade ammonium chloride can occasionally contain a small concentration of nitrate. Check the reagent assay prior to purchase. An alternative buffer recipe is provided in the Lachat Nitrate/Nitrite method.
- 24.12 Lachat Technical Support 1-800-247-7613.
- 24.13 Data backup to CD should be done annually, and noted in the instrument maintenance logbook and the data spreadsheet.

Rev. Date:	Revision #:	Summary of Changes:	Submitted By/Date:	Approved By:	Effective Date:
1/15/2015	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 1/15/2015		

Standard Operating Procedure Signature Page

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

[illegible]