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https://environment.fiu.edu/facilities-research-groups/cache-nutrient-analysis-core/

Standard Operating Procedure
CAChE-NACF SOP-006

Determination of Total Nitrogen in Water

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Revision # 10
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Prepared by and for

Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility

(CAChE-NACF)

Reviewed and approved by:

9/8/23

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1) Scopes and Applications

a) This test procedure covers the determination of total nitrogen (TN) in fresh and saline surface waters. The method is capable of measuring total nitrogen at concentrations between 1.43 µmol/l (0.02 ppm) to 214.2 µmol/l (3.0 ppm) at a precision of 20% relative standard deviation (%RDS) or better, and an accuracy between 90 and 110%. The method is also applicable for analysis of dissolved nitrogen (DN) of filtered water samples.

2) Summary of Method

a) CAChE-NACF follows the ASTM D5176-91(2003) - "Standard Test Method for Total Chemically Bound Nitrogen in Water by Pyrolysis and Chemiluminescence Detection" (the procedure is a modification of the classical Dumas (1831) method of determining nitrogen by a combustion technique with the addition of chemiluminescence). The method involves converting all forms of nitrogen into nitric oxide (NO) upon combustion of a sample with oxygen at a temperature of 1050°C. The NO is reacted with ozone (O_3) to form a metastable form of nitrogen dioxide (NO_2^*). As the metastable form of nitrogen dioxide decays, a quanta of light is emitted in an amount directly proportional to the amount of nitrogen in the sample. The chemiluminescent emission is detected by a photomultiplier tube.

$$\text{R-N} + O_2 \text{ ------> } NO + H_2O + \text{Oxides}$$
$$NO + O_3 \text{ ------> } NO_2^* + O_2 \text{ ------> } NO_2 + hv$$

An ANTEK Instrument, Inc. Model 9000 Nitrogen Analyzer is used to determine TN of a 5 µL injection from a prepared water sample. The instrument is run according to the Installation/Operation/Service Manual provided by ANTEK Instruments, Inc., except that Oxygen gas is used as a carrier gas instead of Argon/Oxygen to promote complete combustion and oxidation of the nitrogen in the water samples. Total nitrogen is determined on unfiltered samples, while total dissolved nitrogen is determined on filtered samples. An auto-sampler is used to inject the samples into the analyzer.

Alternatively, a Trace S/N Cube from Elementar, Inc. is available to determine TN/DN of a 40 µl injection from a preserved water sample. The chemistry and principles of the Trace S/N Cube is the same as the ANTEK-9000, the only difference is an Argon/Oxygen mixture is used as the carrier gas as suggested by the manufacturer.

3) Definition, Quality Control, Calculation and Acceptance Criteria

a) Method detection limit (MDL): The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results. The MDL has been determined according to the EPA procedure described in 40 CFR Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit" - revision 2 (Dec 2016), see Table 1 below.

Analysis	MDL (μmoles/L)		PQL (μmoles/L)		MDL (ppm)		PQL (ppm)	
	SW	FW	SW	FW	SW	FW	SW	FW
TN	3.5		17.86		0.05		0.25	
Updated as of 08/01/2023								

Table 1. MDL for TN analysis.

b) The Practical Quantitation Limit (PQL) is defined as the lowest level that can be reliably achieved within the specified limits of precision and accuracy during routine laboratory operating conditions. CAChE-NACF, following NELAC and SFWMD suggestions, has established the PQL at the S2 value (the lowest non-blank standard) of the run (Table 2 in 10.c). Any value above MDL, but below PQL will be considered a real number, but below quantifiable levels and it will be reported with a qualifier flag denoting that it could not be quantified with certainty; see Table 1 above for current MDL and PQL values.

c) Calibration Standard: Solutions prepared from the primary standard solution or stock standard used to calibrate the instrument response with respect to analyte concentration. The correlation coefficient of the curve must be ≥ 0.995. The relative error (%) of the lowest and the mid-point calibration standard will be calculated. As part of the calibration evaluation, calculation of the Percent Relative Error (%RE) will be performed for two of the analyses' calibration levels (low and midpoint). The Relative Error, at both of these levels, shall meet the criteria of %RE low <20% and %RE mid < %10 and can be calculated using the following equation:

$$RE(\%) = \left(\frac{|X'i - Xi|}{Xi}\right) \times 100$$

Where:
Xi = True value for the calibration standard
X'i = Measured concentration of the calibration standard.

d) Method reagent blanks (MB): analyte-free water (deionized water, DIW) prepared exactly like a sample with a frequency of one per batch (a batch is 20 samples as per TNI guidelines). MB should be no more than the established MDL value.

e) Laboratory sample duplicate: CAChE-NACF runs one sample in duplicate per 25 samples. Sample duplicates should have a Relative Percent Difference (RPD) ≤ 20 %.

f) Initial Calibration Verification (ICV) consisting of one intermediate standard (2.0 ppm) run after the calibration curve. The ICV is prepared from the Primary Standard (8.e.i). The acceptance criterion is 90-110% of the expected value

g) Continuing calibration verification standards (CCV): One intermediate standard (2.0 ppm) prepared with the primary source standard is run at a frequency of every 10 samples. The CCV is prepared from the Primary Standard (8.e.i). The acceptable criterion is 90-110%. At the end of each run a higher standard (S6) is also added as a High CCV (HCCV) and must be within 90-110% recovery of the expected value.

h) Quality Control Sample (QC) (or Laboratory Control Sample-LCS): A solution containing the method analyte of a known concentration, made from a secondary source (different manufacturer or Lot number), that is used to fortify an aliquot of the method blank (sample matrix/reagent blank) to certify that the accuracy of the analytical process is within the expected performance of the method. CAChE-NACF purchases certified reference material standards (CRM) or standards with NIST traceability certificates as the Secondary Standards or QC standard (8.e.ii). These standards will be prepared according to the instructions provided by the company. Based on the results for each analysis, appropriate dilution factors will be determined so that the results fall within the middle range of the calibration for each analysis. The NIST traceable QC standard should be prepared every **90 days**. The acceptance criterion for the QC percent recovery is 90-110%.

i) Matrix spikes samples (MS) and Matrix spikes duplicates (MSD): An aliquot of a sample from the survey (or an S3, not a blank, in the absence of enough sample or in case of high concentration that will go over the calibration curve) that is run un-spiked (UMS) and also is run spiked (MS) with a known concentration quantity (S) of the method analyte. A set of two UMS and two MS is run every 20 samples or sites. The concentration from the un-spiked duplicate is subtracted from the corresponding spiked sample result and compared to the known spike concentration to obtain the % Recovery.

Accuracy is defined as the agreement between the analytical results and the known concentration.

$$MS\ \%R = \left(\frac{MS - UMS \times \left(\frac{V1}{V1 + V2} \right)}{S} \right) \times 100$$

Where:
UMS = concentration of un-spiked sample
MS = concentration of spiked sample
V1 = volume of UMS before spiking (1.5 ml, 8.c)
V2 = volume of MS that spiked into UMS (0.5 ml, 8.c)
S = expected concentration of spike in sample (0.625 ppm)
%R = percent recovery

In the absence of enough sample or if samples are of elevated concentration (in which case spiking the sample would result in a value going over the calibration curve highest standard) the technician can use a low standard (example: C3/S3) as a sub-sample instead. The MS percent recovery acceptance criterion is 80-120%.

Precision is defined as the agreement or closeness of two or more results. MS duplicates are used to calculate precision by calculating their RPD. This is calculated by taking the absolute value of the difference between two MS and dividing it by the average of the two MS and then multiplying it by 100:

The acceptance criterion for RPD is ≤ 20 %.

$$RPD(\%) = \left(\frac{Absolute(MS1 - MS2)}{\left(\frac{MS1 + MS2}{2} \right)} \right) \times 100$$

For method performance evaluation during a long period of time (such as quarter or annual evaluation), precision can be calculated from the Average Deviation of the data set from a quality control standard (i.e. ICV, QC, MS, MSD, CCV) and the Mean of that standard collected during certain time period.

$$Precision\ (\%) = \left(\frac{Average\ Deviation}{Mean}\right) \times 100$$

j) QC cover page: includes all QC data and ranges, specific for every individual run. It also includes the instrument information, project IDs, sample IDs, date of the run, starting time of the analysis, technician identification, sample preparation date. Technician's initials and date of data transfer are recorded in the QC page, as well as the QC reviewer's initials and date of data review. A section is available in the QC page to record any comments pertaining the analytical run and/or data results. See Figure 1 for an example Form (18).

4) Interferences

a) There are no known interferences with this procedure as long as all glassware is cleaned properly, and DIW is used to make the standards. The emission wavelength is completely specific for chemically bound nitrogen; therefore, there is no interference with other compounds. In addition, the method is specific for chemically bound nitrogen and does not detect di-nitrogen (N_2).

b) Care must be taken not to contaminate samples or laboratory glassware. Given the small size of the auto-analyzer vials, cleaning is not practical; therefore, auto-analyzer vials are obtained clean directly from the supplier, used once, and then discarded.

c) All laboratory glassware used for standard preparation is washed with water, soaked in acid at least overnight, thoroughly rinsed with Type I water and air dried. The inside of all glassware, sample bottles, auto-analyzer vials or the auto-analyzer injection needle should not be touched since human contact can contaminate the samples with nitrogen.

5) Safety

a) CAChE-NACF maintains records of required training for safe handling of chemicals. This training is approved and supervised by Florida International University Department of Environmental Health and Safety.

b) Each chemical should be regarded as a potential health hazard, and exposure should be as low as reasonably achievable.

c) Safety Data Sheets (SDS) are available to all personnel involved in the chemical analysis in the department’s server Z:\Serc Lab\SDS - Safety Data Sheets and accessible at all times from any laboratory computer workstation.

d) The following chemicals have the potential to be toxic or hazardous, please consult the SDS:

   i) Hydrochloric acid

6) Equipment and Supplies

a) Balances: Mettler Toledo AE 260-S (serial# G80748), Mettler Toledo ML-104T/00 (serial# B621525835) and Mettler Toledo XSR104 (serial# C231562564) analytical, four place balances.

b) Glassware: Class A

c) ANTEK Instrument, Inc. Model 9000 Nitrogen Analyzer equipped with the following:

A200S auto-sampler, Chemiluminescent Nitrogen Detector (CLD), Pyro Tech Furnace, ozone generator, vacuum pump and accessories.

d) ELEMENTAR Trace S/N Cube Analyzer equipped with the following: built-in auto-sampler, built-in furnace, Horiba Chemiluminescent Nitrogen Detector (CLD), ozone generator and vacuum pump.

e) Oxygen Supply regulated to 20 psi for ANTEK-9000

f) Oxygen Supply regulated to 20 psi and Argon Supply regulated to 20 psi for ELEMENTAR Trace S/N Cube

g) Dry air supply regulated to 10 psi for Nafion driers in ANTEK 9000 and Elementar Trace S/N Cube.

h) Pipettes: 10 µl, 20 µl, 100 µl, 300 µl, 1000 µl, 5000 µl and repeater pipette.

i) Borosilicate glass sample vials (2.0 ml), with teflon/silicone septa seals for the ANTEK-9000 auto-analyzer, and pre-slit teflon/silicone septa seals for the ELEMENTAR Trace S/N Cube.

7) Instrument Parameters

a) ANTEK-9000

Pyrolysis Temperature:	1050°C
Pyrolysis Oxygen Flow:	100 ml/min
Oxygen to Ozone Flow:	25 ml/min
System Pressure:	0.5-0.75 psi
Vacuum:	25 in/Hg
PMT Voltage:	650-900
Gain:	X50 Hi
Sample Volume:	5 µl + 3µl air

b) Elementar Trace S/N Cube

Pyrolysis Temperature:	1050°C
Argon Flow:	279 ml/min
Pyrolysis Oxygen Flow:	65-70 ml/min
Ar/O2 Flow:	350 ml/min
Sample Volume:	40 µl + 5 µl air

8) Reagents and Standards

a) Reagent Blank (RB) and Method Blank (MB): These blanks consist of the same analyte-free water (DIW) used to prepare the working standards and dilutions to establish the baseline or determine the value of analyte free water through the whole analytical procedure.

b) Wash Water: Analyte free water (DIW) placed in the run to monitor baseline or mitigate salt build-up in the system.

c) Matrix Spike Sample Preparation

  i) Matrix Spike working solution: S6 (3ppm) is used as the spiking solution to spike a sample from a given project (or an S3 (0.5 ppm) in the absence of enough sample).

  ii) Each run, or as required by contract, need to have two Matrix Spikes run every 20 samples. When a new set of working standards is prepared (10.a), the technician will spike a series of approximately thirty (30) vials with a sample analyzed during that month (or an S3 in the absence of sufficient sample). The sample is prepared following procedure in section 11.a.i and a duplicate of 1.5 ml of the sample or S3 is spiked with 0.5 ml of the 3.0 ppm Matrix Spike. This spiking solution will yield a final concentration of 0.625 ppm. The percent recovery is calculated by equation in 3.i.

d) Sample preparation reagents

  i) Hydrochloric Acid, 3 N: ACS reagent grade 3 N HCl is made by adding 125 ml of concentrated 12N HCl into a 500 ml volumetric flask and diluting to the mark with DIW. 3N HCl hydrochloric acid is used as a preservative and also to prevent formation of oxide and carbonate salts inside the injection needle when the needle enters the pyrotube. Store at room temperature for **180 days**.
  (Use caution when making acid solutions. Always add acid to water. The mixing of acid in water may generate heat.)

e) TN Standards

  i) Nitrogen Primary Standard, 500 ppm: 0.3612 g of pre-died (1 hour at 105 °C) anhydrous Potassium Nitrate (KNO_3) is dissolved in 100 ml of DIW in a volumetric flask. Preserve with 1 ml of chloroform and store at room temperature for **180 days**.

  ii) Nitrogen Secondary Standard: NIST traceable standard or Certified Reference Material (CRM). The proper storage method and shelf life are referred to the manufacture instruction.

9) Sample Storage

a) Sample bottles are stored in refrigerators at 2-6 °C. Samples have a holding time of 28 days from sample collection time. Storage of samples is in refrigerators designated for sample storage only.

10) Calibration and Standardization

a) Working standards are prepared every **30 days** by dilution of the primary standard (8.e.i) in DIW. The working standards are made to bracket the expected concentration of the samples as shown in Table 2 below. A logbook is kept by the ANTEK-9000 and ELEMENTAR Cube to record date and name of the run, sample ID, the slope of the calibration curve, and the technician's initials. A separate logbook is used to record the preparation dates of the TN standards and the reagents.

b) Transfer approximately 1.5 ml of working standards to the glass auto-analyzer vials and proceed to acidify as indicated in section 11.a. Acid preserved working standards are good for **30 days** at room temperature.

c) Calibration curve: Calibration curve is not required on each analytical run. According to 2016 NELAC Standard (section 5.5.5.10), when an initial instrument calibration is not performed on the day of analysis, the validity of the initial calibration shall be verified prior to sample analyses by continuing instrument calibration verification with each analytical batch. All QC criteria, including MB (8.a), ICV (3.f), QC (3.h), UMS and MS (3.i), need to be met prior to sample analysis. CCV's (3.g) will be used to monitor the run. If any of the QC criteria or CCV failed to meet the QC criteria outlined in section 3 of this document or whenever it is expected that the analytical system may be out of calibration, or any instrument setting that relates to calibration is changed, a new calibration should be performed to generate a new calibration curve.

d) Any sample result that falls outside the Linear Dynamic Range (LDR, 13.a.iv) will be diluted and re-analyzed.

Working Standard	**Volume of Primary Standard (µl) added to bring to 100 ml**	**Concentration of N in ppm (and µM)**
S1	0	0.00 (0.0)
S2	50	0.25 (17.8)
S3	100	0.50 (35.7)
S4	200	1.00 (71.4)
S5	400	2.00 (142.5)
S6	600	3.00 (214.2)

Table 2. Preparation for working standard

11) Procedure

a) Sample Preparation

i) Approximately 1.5 mL of sample is transferred into a glass auto-analyzer sample vials in sample trays as shown in Figure 2 (18). Then they will be acidified with 10 µl of 3 N HCl (8.d.i). The vial is sealed with a teflon/silicone lined cap and stored at room temperature until analyzed. When preserved by acid, samples have a shelf life of **90 days** without deterioration; however, sample analysis within 28 days is recommended. Sample preparation is the same for all standards, samples, controls, MB and DIW vials (DIW is used at different points in the run to rinse the equipment and to monitor instrument baseline)

12) Analysis

a) Combustion of the sample and its reaction with ozone is completed by the ANTEK Instrument, Inc. Model 9000 Nitrogen Analyzer or Elementar Trace S/N Cube. Both instruments are programed to perform a triplicate analysis of each sample. The instrument diagram for ANTEK-9000 is shown in Figure 3 (18), and the instrument diagram for Elementar Trace S/N Cube is shown in Figure 4 (18).

b) Each run of the auto-analyzer is set up per batch (20 samples) as follows:

- One or two DIW - wash (run in)
- Calibration curve standards when needed (S1 to S6 described in Table 2)
- Initial S5 ICV, which is prepared from the same source as the S5 from the curve

- QC sample (3.h) - one per 20 samples
- MB (method blank) - two per 20 samples
- Two UMS (8.c)
- Two MS (8.c)
- First set of 10 samples
- DIW and S5 CCVs (3.g) are run every 10 samples to monitor intra-run calibration drifts
- All runs include an S6 HCCV (3.g) at the end

13) Method Performance

a) The following QA/QC assessment tools are used to determine the method performance:

   i) Demonstration of Capability and instrument performance: Refer to Appendix in the CAChE-NACF Quality Manual.

   ii) QC standard check from Proficiency testing at least twice a year or every 7 months. Currently CAChE-NACF is participating, submitting and obtaining reports from Environmental Resource Associates (ERA), A Waters Company, 16341 Table Mountain Pkwy, Golden, CO, 80403.

   iii) Linear Calibration range: every run includes a calibration curve with 6 standards. The analysis of the calibration curve is performed to ensure that the resulting curve is linear and within the acceptance criteria.

   iv) On a quarterly basis, the technicians will include a set of two Linear Dynamic Range (LDR) standards at a concentration of the S6 plus 10% to make sure the method is linear past the highest standard, yet CAChE-NACF requires all samples reading higher than 110% of the highest standard of the curve need to be diluted. The Linear Dynamic Range will be determined annually based on the quarterly collected data.

   v) For QC acceptance criteria refer to section 3 of the SOP- “**Definition, Quality Control, Calculation and Acceptance Criteria”**

   vi) The degree of uncertainty on method performance will be established once sufficient internal performance data is collected, from QC checks, Matrix Spikes Recoveries and Method Blanks. These data will be used to establish control charts and will help establish an on-going precision, or margin of error, statement.

   For method performance evaluation during a long period of time (such as quarter or annual evaluation), uncertainty (such as quarter or annual evaluation) is calculated from 2 times of the Standard Deviation of the data set from a quality control standard (i.e., ICV, QC, CCV) and the Mean of that standard collected during certain time period.

$$Uncertainty\ (\%) = \left(\frac{2 \text{ x } Standard\ Deviation}{Mean}\right) \times 100$$

   vii) For data analysis, validation, calculations and report refer to the CAChE-NACF QA plan section 9.

viii) For laboratory corrective actions refer to CAChE-NACF QA manual, section 10.

ix) Data Qualifiers

(1) G: when the value is detected at or above the MDL in both the sample and the associated field, equipment or trip blank, and the blank value is greater than 10% of the associated samples value.

(2) I: The reported value is greater than or equal to the laboratory method detection limit, but less than the laboratory practical quantitation limit. MDL < Value < PQL

(3) J: Value outside the stablished criteria.

(4) O: Sampled, but analysis lost or not performed.

(5) Q: Sample held beyond acceptable holding time.

(6) T: Value reported is less than the laboratory method detection limit. Value < MDL

(7) U: If component was not detected, then use less than MDL instead of a reported value. If value is reported then use "T"

(8) Y: The laboratory analysis was from an improperly preserved sample. The data may not be accurate.

(9) ? : Data rejected and should not be used.

(10) *: Not reported due to interference.

14) Auto-analyzer Preventative Maintenance

a) Once a week or as necessary, the combustion column of the ANTEK-9000 needs to be cleaned. The oxygen gas is shut down, the vacuum pump is turned off, the nafion dryer air flow is turned off, and the temperature of the furnace is set to room temperature to let the instrument cool down. The combustion column is then taken out of the furnace. The quartz column itself, the inner ceramic column and the ceramic packing pieces are rinsed thoroughly with DIW (sonicate the packing pieces if necessary to remove salts), and all parts are dried in the oven.

b) Elementar Trace S/N Cube does not require frequent column clean-up since the instrument contains a quartz ash finger inside the column to catch combustion residues and salts from the sample matrix. The quartz ash finger needs to be replaced every 1000 injections or as needed.

15) Pollution Preventions

a) Sample preparation and sample analysis are conducted in separate areas of the laboratory.

b) Reagents and samples are stored separately.

c) SDS are available to all personnel for proper management and safe use of chemicals.

16) Waste Management

a) All samples and reagents residuals are discarded according to Florida International University Department of Environmental Health and Safety regulations. All CAChE-NACF laboratories undergo annual inspections and certifications

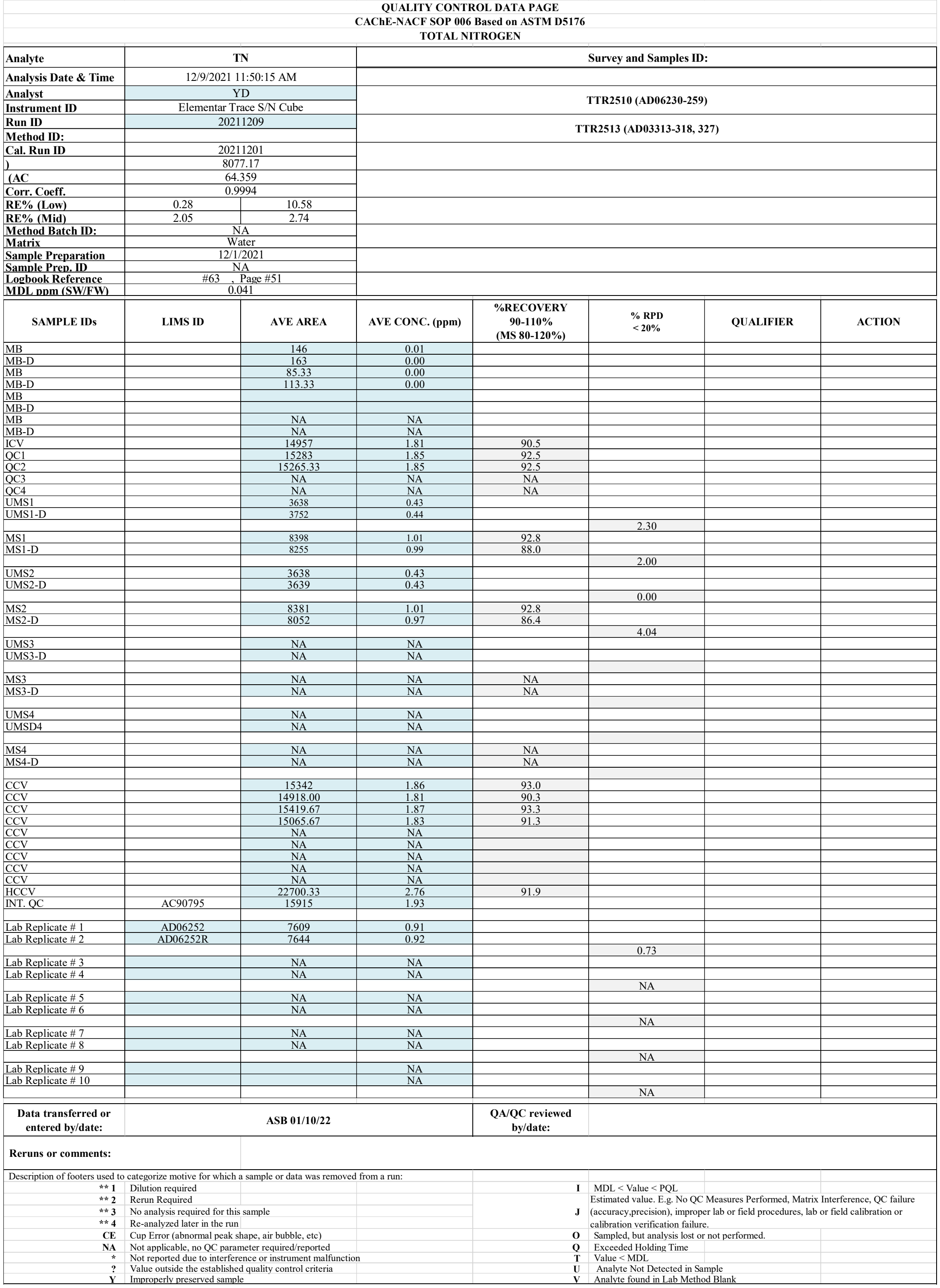
17) References

a) ASTM D5176-91(2003), Standard Test Method for Total Chemically Bound Nitrogen in Water by Pyrolysis and Chemiluminescence Detection, ASTM International, West Conshohocken, PA, 2003, www.astm.org

b) EPA 821-R-16-006: Definition and Procedure for the Determination of the Method Detection Limit, revision 2, Dec. 2016 (e-CFR title 40 part 136, App. B)

c) 2016 NELAC Standard

18) Figures



QUALITY CONTROL DATA PAGE
CAChE-NACF SOP 006 Based on ASTM D5176
TOTAL NITROGEN

Analyte	**TN**		**Survey and Samples ID:**
Analysis Date & Time	12/9/2021 11:50:15 AM		
Analyst	YD		**TTR2510 (AD06230-259)**
Instrument ID	Elementar Trace S/N Cube		
Run ID	20211209		**TTR2513 (AD03313-318, 327)**
Method ID:			
Cal. Run ID	20211201		
)	8077.17		
(AC	64.359		
Corr. Coeff.	0.9994		
RE% (Low)	0.28	10.58	
RE% (Mid)	2.05	2.74	
Method Batch ID:	NA		
Matrix	Water		
Sample Preparation	12/1/2021		
Sample Prep. ID	NA		
Logbook Reference	#63 , Page #51		
MDL ppm (SW/FW)	0.041		

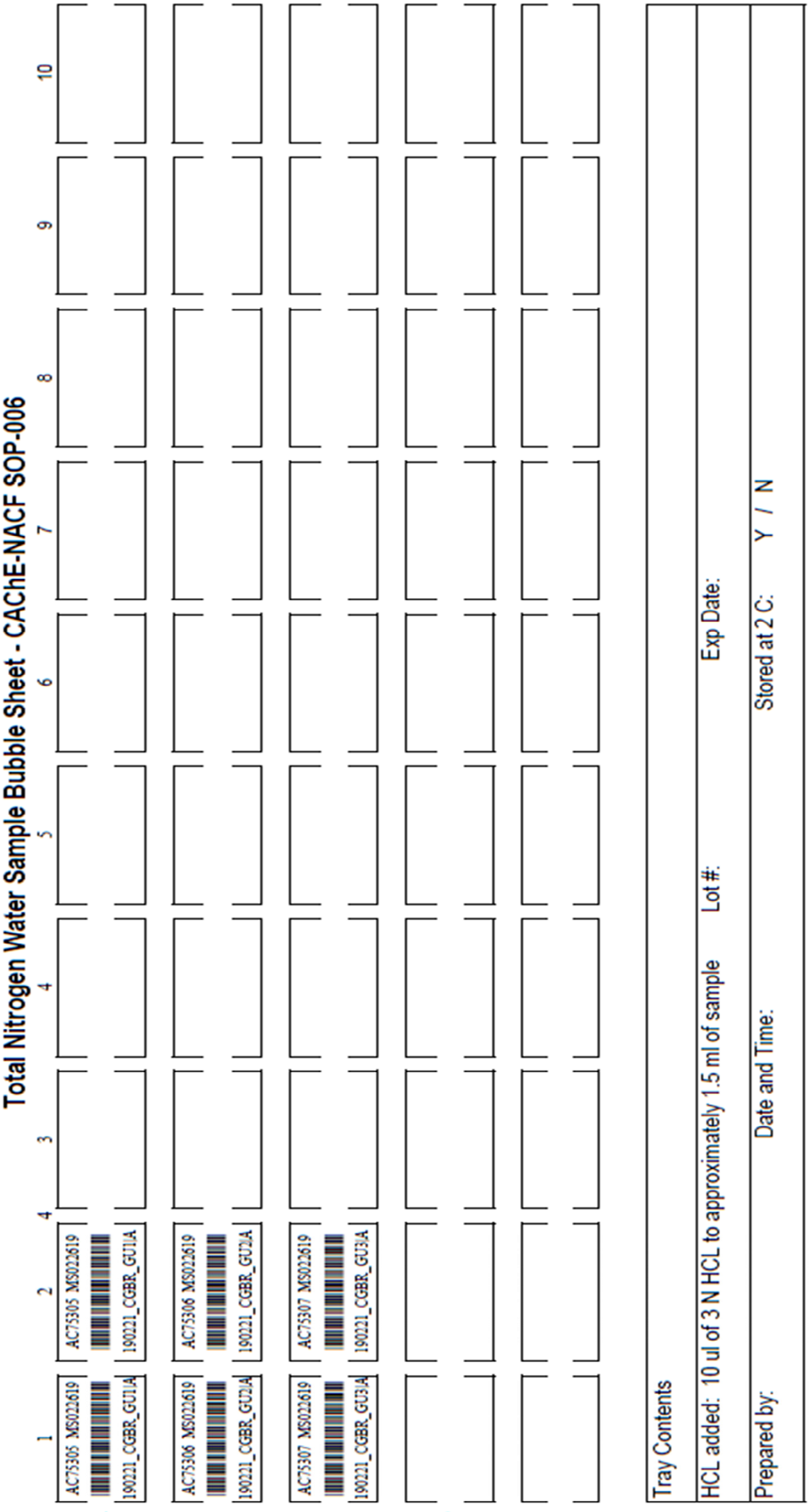
SAMPLE IDs	**LIMS ID**	**AVE AREA**	**AVE CONC. (ppm)**	**%RECOVERY 90-110% (MS 80-120%)**	**% RPD < 20%**	**QUALIFIER**	**ACTION**
MB		146	0.01				
MB-D		163	0.00				
MB		85.33	0.00				
MB-D		113.33	0.00				
MB							
MB-D							
MB		NA	NA				
MB-D		NA	NA				
ICV		14957	1.81	90.5			
QC1		15283	1.85	92.5			
QC2		15265.33	1.85	92.5			
QC3		NA	NA	NA			
QC4		NA	NA	NA			
UMS1		3638	0.43				
UMS1-D		3752	0.44				
					2.30		
MS1		8398	1.01	92.8			
MS1-D		8255	0.99	88.0			
					2.00		
UMS2		3638	0.43				
UMS2-D		3639	0.43				
					0.00		
MS2		8381	1.01	92.8			
MS2-D		8052	0.97	86.4			
					4.04		
UMS3		NA	NA				
UMS3-D		NA	NA				
MS3		NA	NA	NA			
MS3-D		NA	NA	NA			
UMS4		NA	NA				
UMSD4		NA	NA				
MS4		NA	NA	NA			
MS4-D		NA	NA	NA			
CCV		15342	1.86	93.0			
CCV		14918.00	1.81	90.3			
CCV		15419.67	1.87	93.3			
CCV		15065.67	1.83	91.3			
CCV		NA	NA				
CCV		NA	NA				
CCV		NA	NA				
CCV		NA	NA				
CCV		NA	NA				
HCCV		22700.33	2.76	91.9			
INT. QC	AC90795	15915	1.93				
Lab Replicate # 1	AD06252	7609	0.91				
Lab Replicate # 2	AD06252R	7644	0.92				
					0.73		
Lab Replicate # 3		NA	NA				
Lab Replicate # 4		NA	NA				
					NA		
Lab Replicate # 5		NA	NA				
Lab Replicate # 6		NA	NA				
					NA		
Lab Replicate # 7		NA	NA				
Lab Replicate # 8		NA	NA				
					NA		
Lab Replicate # 9			NA				
Lab Replicate # 10			NA				
					NA		

Data transferred or entered by/date:	**ASB 01/10/22**	**QA/QC reviewed by/date:**	
Reruns or comments:			

Description of footers used to categorize motive for which a sample or data was removed from a run:

**** 1**	Dilution required	**I**	MDL < Value < PQL
**** 2**	Rerun Required	**J**	Estimated value. E.g. No QC Measures Performed, Matrix Interference, QC failure (accuracy,precision), improper lab or field procedures, lab or field calibration or calibration verification failure.
**** 3**	No analysis required for this sample		
**** 4**	Re-analyzed later in the run		
CE	Cup Error (abnormal peak shape, air bubble, etc)	**O**	Sampled, but analysis lost or not performed.
NA	Not applicable, no QC parameter required/reported	**Q**	Exceeded Holding Time
*****	Not reported due to interference or instrument malfunction	**T**	Value < MDL
?	Value outside the established quality control criteria	**U**	Analyte Not Detected in Sample
Y	Improperly preserved sample	**V**	Analyte found in Lab Method Blank

Figure 1. TN QC cover page example 1



Total Nitrogen Water Sample Bubble Sheet - CAChE-NACF SOP-006

	1	2	3	4	5	6	7	8	9	10
A	AC75305 MS022619 190221_CGBR_GU1A	AC75305 MS022619 190221_CGBR_GU1A								
B	AC75306 MS022619 190221_CGBR_GU2A	AC75306 MS022619 190221_CGBR_GU2A								
C	AC75307 MS022619 190221_CGBR_GU3A	AC75307 MS022619 190221_CGBR_GU3A								
D										
E										

Tray Contents		
HCL added: 10 ul of 3 N HCL to approximately 1.5 ml of sample	Lot #:	Exp Date:
Prepared by:	Date and Time:	Stored at 2 C: Y / N

Figure 2. TN sample preparation sheet

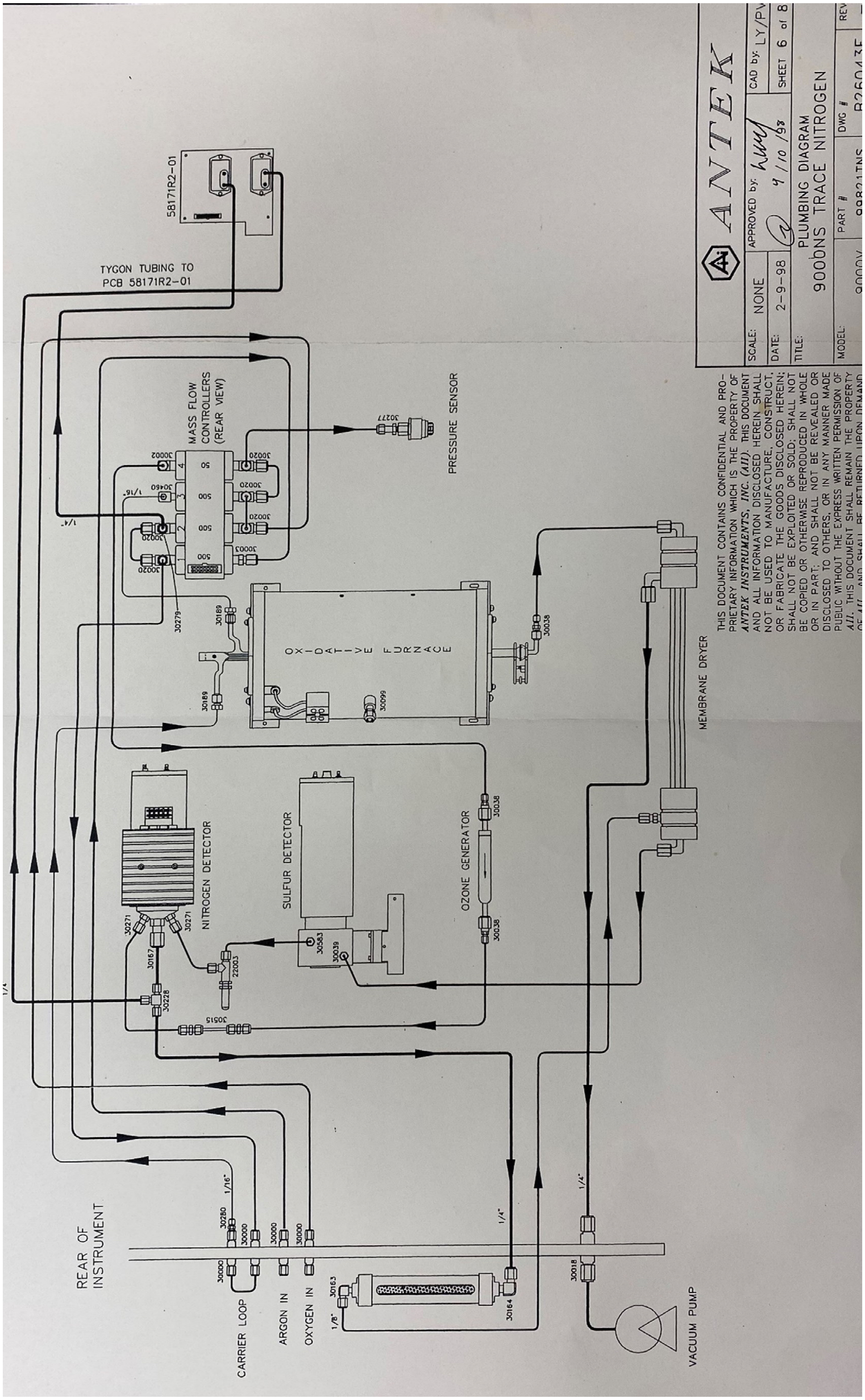
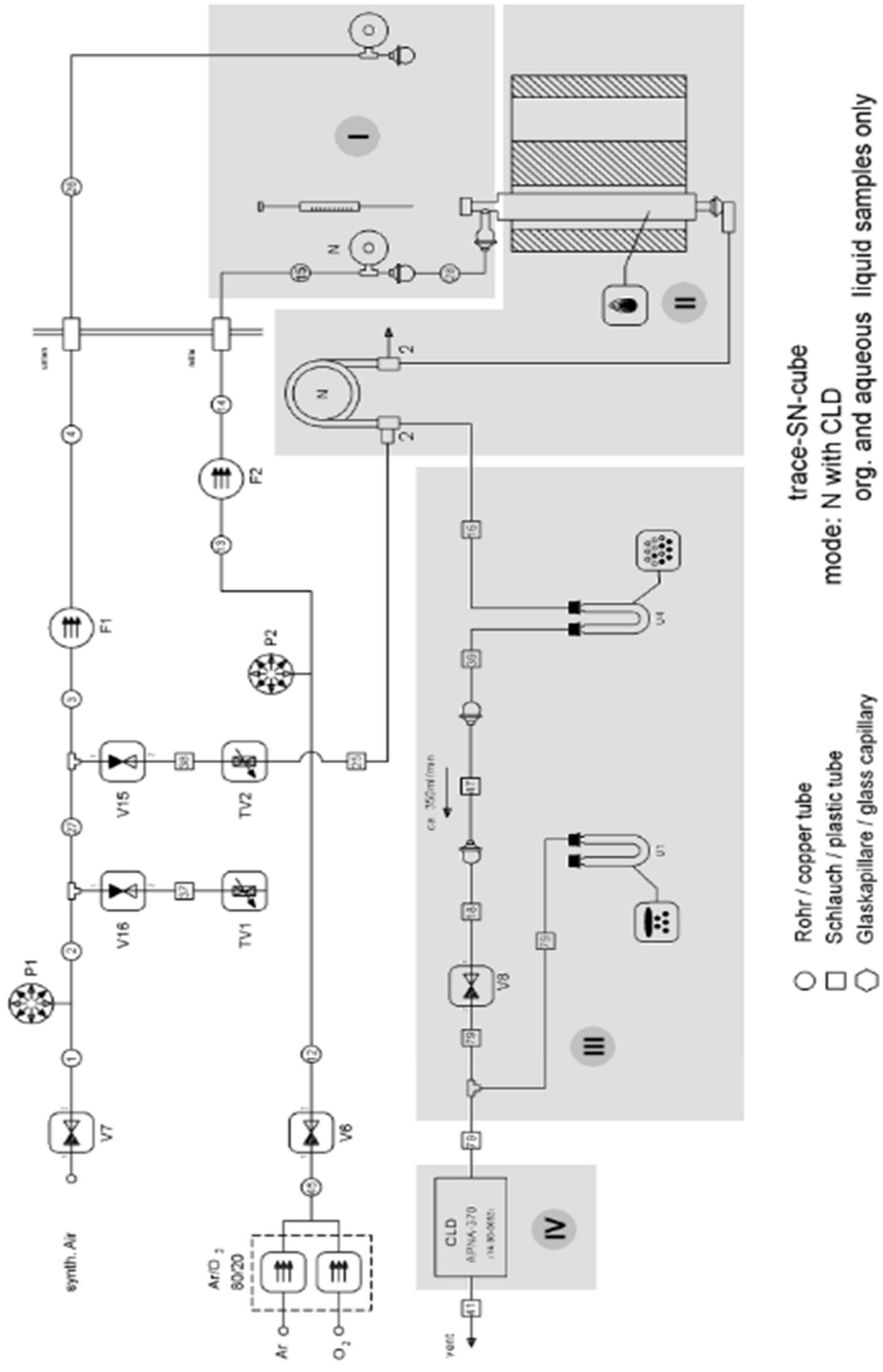


Figure 3. Instrument diagram for ANTEK-9000



Note: In N modes with manual sample injection the combustion tube is located in the right heater.

Figure 4. Instrument diagram for Elementar Trace S/N

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Center for Aquatic Chemistry and Environment
Nutrient Analysis Core Facility
Document Change Log

Determination of Total Nitrogen in Water

Document ID	**CAChE-NACF SOP-006**
Issue Date	11/05/1998
Revision History	**Effective Date**
Revision # 6	11/23/2015
Revision # 7	01/31/2018
Revision # 8	07/01/2019
Revision # 9	05/01/2022
Revision # 10	09/18/2023

Document	**Document Changes**	**Author (s)**
SOP Rev.10	Pg. 3. In section 2, the purpose of the use of Oxygen gas instead of Argon/Oxygen was revised from “to promote complete recovery of the nitrogen in the water samples” to “promote complete combustion and oxidation of the nitrogen in the water samples”. Pg. 4. MDL values were updated in Table 1. Pg. 4 and Pg.5. The following typos were corrected: - Section 3.b: (Table 2 in 10.a) to (Table 2 in 10.c) - Section 3.h: QC standard (8.e) to QC Standard (8.e.ii) Pg. 4. ADM_220711 was included in the Calibration section 3.c. “As part of the calibration evaluation, calculation of the Percent Relative Error (%RE) will be performed for two of the analyses calibration levels (low and midpoint). The Relative Error, at both of these levels, shall meet the criteria of %RE low <20% and %RE mid < %10 and can be calculated using the following equation: $RE(\%) = \left(\frac{\lvert X'i - Xi \rvert}{Xi}\right) \times 100$ Where: Xi = True value for the calibration standard X'i = Measured concentration of the calibration standard. Pg. 5. ADM_221101B was included in section 3.h. Quality Control (Secondary Standard): “CAChE-NACF purchases certified reference	IL YD MK

material standards (CRM) or standards with NIST traceability certificates as the Secondary Standards or QC standard. These standards will be prepared according to the instructions provided by the company. Based on the results for each analysis, appropriate dilution factors will be determined so that the results fall within the middle range of the calibration for each analysis".

Pg. 6. In order to assess method precision, the following statement was included in section 3.i.:
"For method performance evaluation during a long period of time (such as quarter or annual evaluation), precision can be calculated from the Average Deviation of the data set from a quality control standard (i.e. ICV, QC, MS, MSD, CCV) and the Mean of that standard collected during certain time period."

$$Precision\ (\%) = \left(\frac{Average\ Deviation}{Mean}\right) \times 100$$

Pg. 6. Addition of new pipette (300 µl) and balance to Section 6. (Equipment and Supplies).

Pg. 7. Insertion of dry air supply regulated to 10 psi for Nafion driers in ANTEK 9000 and Elementar Trace S/N Cube in section 6.g

Pg. 7. Removal of Eppendorf pipette from section 6.h.

Pg. 7. Update Pyrolysis Oxygen flow in section 7.a (ANTEK) from 220 ml/min to 100 ml/min.

Pg. 8. Replacement of the Mixed QC Standards in sections 8.e.ii, for NIST traceable or CRM standard to be used as Secondary Standard (QC Standard) as a result of ADM_221101B

Pg. 8. QC standard preparation was deleted from section 8.e.ii since the lab now uses the NIST traceable Secondary Standard (QC Standard) which is prepared according to the manufactures' specifications.

Pg. 9. The following statement was added to Section 10 (Calibration and Standardization): "Any sample result that falls outside the Linear Dynamic Range (LDR, 13.a.iv) will be diluted and re-analyzed."

Pg. 9. In order to assess method uncertainty, the following statement was included in section 13.a.vi.:

"For method performance evaluation during a long period of time (such as quarter or annual evaluation), uncertainty (such as quarter or annual evaluation) is calculated from 2 times of the Standard Deviation of the

	data set from a quality control standard (i.e., ICV, QC, CCV) and the Mean of that standard collected during certain time period.” $$Uncertainty\ (\%) = \left(\frac{2 \text{ x } Standard\ Deviation}{Mean}\right) \times 100$$ Pg. 10. Linear Calibration range in section 13.a.iii was rephrased to “The analysis of the calibration curves is performed to ensure that the resulting curve is linear and within the acceptance criteria”. Pg. 10. The following reference section was corrected: • Section 12.b: QC sample (8.e.iii) to QC sample (3.h) Pg. 11. Description for the Data Qualifier “J” in section 13.a.ix.3 was modified form “Estimated Value, qualifier has to be accompanied by detailed explanation. Value outside the established criteria” to “Value outside the established criteria”. Pg.12. Addition of the 2016 NELAC Standard as a reference in Section 17.	
ADM_221101B	Quality Control Standard or Secondary Standard used for the analysis will be purchased from a NIST traceable provider and prepared according to the provider’s instructions.	IL
ADM_220711	Calculation of Percent Relative Error (%RE) for two of the analysis calibration’s points	IL
SOP Rev.9	Pg.3, MDL definition is updated to “The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results”. Pg.3, MDL values were updated to currents ones (released 07/01/21) in table 1 Pg.3, Additional sentence was added to clarify that the ANTEK instrument is only used as a backup. Pg.5, Percent recovery ranges for Matrix Spike were updated to 80-120% Pg.10, Additional sentence was added to clarify that the LDR is determined annually based on the quarterly collected data.	IL YD MK

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September 8th, 2023

Purpose: Document Acknowledgement Receipt
Document: Determination of Total Nitrogen in Water
SOP ID: CAChE-NACF SOP-006 Rev.10
Prepared by: CAChE-Nutrients Analysis Core Facility
Effective Date: September 18th, 2023

I certify that I have received, read and agree to follow the procedures defined in the CAChE-NACF SOP-006 Rev. 10

Name	Signature	Date
Stephanie Pericles	Stephanie Pericles	9-12-23
Pedro Lorenzo	Pedro Lorenzo	09-12-23
Isel Anarela		9/13/23
Jose Viña		9/03/24

Name	Signature	Date
Name	Signature	Date
Name	Signature	Date
Name	Signature	Date
Name	Signature	Date
Name	Signature	Date

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November 11, 2024

Purpose: To update SOP-006 by removing references to an obsolete TN analyzer (ANTEK).

Prepared by: Ingrid Ley

Effective Date: November 11, 2024

Amendment ADM_241111

This amendment removes any reference to the obsolete ANTEK Instrument Model 9000 Nitrogen Analyzer from SOP-006. Any reference to the old instruments is no longer valid since the instrument is no longer in service.

Approved by

11/11/24

Dr. Yan Ding, Director

Date

11/11/2024

Ingrid M. Ley, QA/QC Officer

Date

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March 21st, 2025

Purpose: To update SOP-006 by including a new Total Nitrogen Analyzer Instrument

Prepared by: Ingrid Ley

Effective Date: March 24th, 2025

Amendment ADM_250321

This amendment updates the SOP-006 to include the use of the instrument Xpert TNb Custom (TN Analyzer) with Archie Autosampler from the company Trace Elemental Instruments. All procedures and quality control steps outlined in the SOP shall be applied to this instrument as appropriate.

MDLs have been performed for this instrument and they have met the required criteria established in the EPA's Method Detection Limit (MDL) Procedure, Revision 2. While the new instrument MDL was lower than the existing MDL, the current MDL value for TN will be retained for consistency.

The new instrument is now approved for use in routine analysis as outlined in the SOP.

Approved by

Dr. Yan Ding, Director

03/21/2025
Date

Ingrid M. Ley, QA/QC Officer

03/21/2025
Date