FIU | Center for Aquatic Chemistry and Environment
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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 Any reference to the old instruments is no longer valid since the instrument is no longer in service.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

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| Dr. Yan Ding, Director | 11/11/24<br>Date |
| Ingrid M. Ley, QA/QC Officer | 11/11/2024<br>Date |

FIU Center for Aquatic Chemistry and Environment
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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** |
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| Issue Date | 11/05/1998 |
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| **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".<br>Pg. 4. MDL values were updated in Table 1.<br>Pg. 4 and Pg.5. The following typos were corrected:<br>• Section 3.b: (Table 2 in 10.a) to (Table 2 in 10.c)<br>• Section 3.h: QC standard (8.e) to QC Standard (8.e.ii)<br>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:<br>$RE(\%) = \left(\frac{\lvert X'i - Xi \rvert}{Xi}\right) \times 100$<br>Where:<br>Xi = True value for the calibration standard<br>X'i = Measured concentration of the calibration standard.<br>Pg. 5. ADM_221101B was included in section 3.h. Quality Control (Secondary Standard): "CAChE-NACF purchases certified reference | IL<br>YD<br>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

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| | data set from a quality control standard (i.e., ICV, QC, CCV) and the Mean of that standard collected during certain time period.”<br>$Uncertainty\ (\%) = \left(\frac{2\ \text{x}\ Standard\ Deviation}{Mean}\right) \times 100$<br>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”.<br>Pg. 10. The following reference section was corrected:<br>• Section 12.b: QC sample (8.e.iii) to QC sample (3.h)<br>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”.<br>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”.<br>Pg.3, MDL values were updated to currents ones (released 07/01/21) in table 1<br>Pg.3, Additional sentence was added to clarify that the ANTEK instrument is only used as a backup.<br>Pg.5, Percent recovery ranges for Matrix Spike were updated to 80-120%<br>Pg.10, Additional sentence was added to clarify that the LDR is determined annually based on the quarterly collected data. | IL<br>YD<br>MK |

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Method Summary of
CAChE-NACF SOP-006, Rev. 10
Prepared for Battelle NEON program

# Determination of Total Nitrogen in Water

Effective date 09/18/2023

Prepared by

Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility

(CAChE-NACF)

## 1) 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.

## 2) Sample Storage

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

## 3) Sample Preparation

i) Approximately 1.5 mL of sample is transferred into a glass auto-analyzer sample vials in sample trays. Then they will be acidified with 10 µl of 3 N HCl. 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)

## 4) Analysis

Each analytical batch (20 samples) include:

- One or two DIW - wash (run in)
- Calibration curve standards when needed (daily calibration curve is not required if QC requirements are met)
- Initial calibration verification standard (ICV), quality control standard (secondary source, QC), method blank (MB), matrix spike and duplicate (UMS, UMSD, MS, MSD)
- First set of 10 samples
- Samples are bracketed by continuing calibration verification standards (CCVs). Each bracket includes no more than 10 samples plus one analytical replicate at the end of each bracket and one MB. CCVs and RBs are run every 10 samples to monitor baseline and intra-run calibration drifts.

## 5) Quality Control/Assurance System

Quality checks are evaluated as described in the NEON SOW