Method for Nitrate Reductase Nitrate-Nitrogen Analysis of Drinking Water Version 1.0 Revision 1.0 15December, 2015

1. Scope and Application

1.1 This test method is applicable to the determination of nitrate plus nitrite (as nitrogen) in drinking water.

1.2 The standard range of this test method is from 0.05 to 5 mg/L of nitrate-nitrogen. Samples with greater than 5 mg N/L may be diluted with deionized water (nitrate-free) to bring them into the range of the test method. In addition, for samples with nitrate-N content lower than 0.05 mg N/L, the sample volume used in the test may be increased for a low range test method from 0.01 to 1.0 mg N/L.

1.3 Quality Control and Quality Assurance (QC/QA) procedures and criteria are delineated. Precision and Bias of the method has been validated to be ±10% for all QC procedures and samples of a variety of Drinking Water matrices.

1.4 This description of the test method does not purport to address all safety concerns associated with its use. It is the responsibility of the user of this test method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2. Summary of Method

2.1 Reduction of nitrate in the sample is accomplished by using eukaryotic nitrate reductase (EC 1.1.7.1-3; NaR) to catalyze the conversion of nitrate to nitrite in the presence of NADH or NADPH as reductant, in a biochemical buffer with a pH near neutrality (Campbell et al., 2006). This version of the Method is for discrete analyzers.

2.2 The combined nitrite (both original nitrite and that generated by reduction of nitrate to nitrite) is reacted with sulfanilamide and subsequent coupling with N-(1-napthyl)ethylenediamine dihydrochloride to yield a pink color. The absorbance of the chromophore is measured at ~540 nm in a spectrophotometer.

2.3 Calibrants of certified nitrate standard are treated with the reduction step and colorizing reaction to yield a standard curve which is used to determine the nitrate-N content of samples, expressed as mg/L nitrate N. This step insures that NaR is functioning.

2.4 Nitrite content of the sample (expressed as mg/L nitrite-N) can be determined by omitting the reduction step and directly reacting the sample with the color dyes.

2.5 Finally, nitrate-N content can be calculated by subtracting the nitrite content (mg/L nitrite N) from the combined nitrate plus nitrite (mg/L nitrate+nitrite N), to yield the nitrate content (expressed as mg/L nitrate N).

3. Definitions

3.1 Nitrate Reductase (NaR): NADH:NaR (EC1.7.1.1 and CAS 9013-03-0) with 1 unit of enzyme activity defined as 1 µmol nitrite produced per minute at 30°C and pH 7 with NADH (refer to section 3.4) as electron donor. See Note following Section 7.6 for information on the type of NaR acceptable for this method.

3.2 Discrete Analyzer: a programmable, computer-controlled instrument with colorimetric detector that automates wet chemical analysis by using one or more robotic arms interfaced to high-precision volumetric dispensers to aspirate and dispense samples, standards, diluents and reagents.

3.3 Greiss Reaction: chemical formation of an azo-dye by diazotization of nitrite ion with sulfanilamide (CAS 63-74-1), at an acidic pH, and subsequent coupling with N-(1-naphthyl)ethylenediamine hydrochloride (CAS 1465-25-4). The product of this reaction is a dye that absorbs light at 540 ± 20 nm.

3.4 NADH: β-Nicotinamide adenine dinucleotide, reduced form (CAS 53-54-9), is a coenzyme found in all living cells.

3.5 EDTA: Disodium ethylenediaminetetraacetate dihydrate (CAS 60-00-4, a heavy metal chelator.

4. Interferences

4.1 Sample color that absorbs at wavelengths between 520 and 560 nm interferes with the absorbance measurements. When color is suspect, analyze a sample blank, omitting the N-(1-naphthyl)ethylenediamine dihydrochloride from the color reagent. **NOTE:** The instrumentation described in this test method may automatically correct for some turbidity and sample color.

4.2 Certain ions may cause interferences. However, the inclusion of EDTA in the system's phosphate buffer (section 3.6 and 7.1) overcomes most, if not all, of these heavy metal interferences.

5. Safety

5.1 General laboratory safe practices should be used in handling all samples and reagents in this test method.

5.2 When preparing the sulfanilamide color reagent in hydrochloric acid (section 7.4), the user should handle the concentrated hydrochloric acid with extra caution and wear heavy gloves and eye protection. The prepared sulfanilamide color reagent should also be handled with caution since it is ~3 N hydrochloric acid.

5.3 Potassium nitrite is toxic if swallowed, and laboratory tests suggest that it may be mutagenic or teratogenic. Gloves and safety glasses are used when handling potassium nitrite.

5.4 Potassium nitrate, unlike potassium nitrite, is not toxic to humans, unless one is chronically exposed to nitrate such as in your drinking water or food. Potassium nitrate will not explode on its own. However, it is recommended that standard laboratory safety practices such as the use of safety glasses and gloves be used when handling it.

6. Equipment

6.1 Automated Discrete Analysis System (see section 3.2).

6.2 "Discrete photometric analyzers provide excellent analytical performance for colorimetric, enzymatic, and electrochemical measurements in either a compact tabletop or stand-alone design. Analyzers cover a wide range of applications including food and beverage analysis, water and soil testing, and industrial quality control. With its automated operating system, laboratories can measure multiple analytes simultaneously while reducing total analysis time." See: http://www.thermoscientific.com/content/tfs/en/products/automated-discrete-analyzers

6.3 Discrete Analyzer Characteristics

a. Detectors: Colorimetric detector with multiple optical interference filters built into a filter wheel (8–12 wavelength) Or Spectrophotometer (27–30 wavelengths or scanning)

b. Sample delivery system: High-precision syringe or piston pump

c. Heater: Resistant or Peltier-based heater

d. Dilution device: Built-in dilution function

e. Process: Individual cuvette, sequential batch analysis or random-access single samples

f. From http://www.oico.com/documentlibrary/2235app.pdf

6.4 Currently available DA instruments are:

a. Thermoscientific Instruments see: http://www.thermoscientific.com/content/tfs/en/products/automated-discrete-analyzers

i. Aquakem™ Photometric Analyzers: Models 200, 250, & 600

ii. Arena™ Photometric Analyzers: Models 30, 60, 20, & 20XT

iii. Gallery™ and Gallery™ Plus Automated Photometric Analyzers

b. Unity Scientific see: http://www.unityscientific.com/products/wet-chemistry

i. SMARTCHEM® 200 and SMARTCHEM® 170 ; EASYCHEM® Plus

ii. Westco Scientific Instruments see: http://westcoscientific.com/

iii. SMARTCHEM® 200 and SMARTCHEM® 170 ; EASYCHEM® Plus

7. Reagents and Standards

7.1 Phosphate Buffer Solution: Dissolve 3.75 g of potassium dihydrogen phosphate (KH_2PO_4; CAS 7778-77-0), 0.01 g of disodium ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$), and 1.4 g potassium hydroxide (KOH; CAS 1310-58-3) in about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. Transfer this solution to a screw-cap container and store at 4°C. This solution is stable for 6 months.

7.2 β-nicotinamide adenine dinucleotide, reduced form (NADH) stock solution (2mg/mL): dissolve 0.1 g NADH in 25 mL of reagent water contained in a 50 mL volumetric flask, dilute to the mark and mix. Transfer 1-mL aliquots to 1.5 mL snap-cap colorless polypropylene vials and store at -20°C. Stable for 1 month. **NOTE:** NADH is a hygroscopic white powder that is freely soluble in water. The solids are stable if stored dry and protected from light. Neutral solutions are colorless and stable for 1 week if stored at 4°C, but decompose rapidly under basic or acidic conditions.

7.3 NADH working solution: thaw one 1-mL vial of NADH stock (refer to section 7.2) and dilute to 10 mL with phosphate buffer (refer to section 7.1). This reagent is stable for about 8 hours. Prepare sufficient NADH working solution for the number of samples and standards to be analyzed. **NOTE**: NADH inhibits color formation in the Greiss reaction (refer to section 3.3). The molar concentration of NADH in the reduction medium should be about twice that of the highest calibration standard.

7.4 Sulfanilamide (SAN) Reagent (10g/L): While stirring constantly add 300 mL of concentrated hydrochloric acid (HCl, 37% w/v)) and 10 g of sulfanilamide to about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. This solution is stable for about six months when stored in a brown bottle at 20°C.

7.5 N-(1-naphthyl)ethylenediamine dihydrochloride (NED) solution (1g/L): dissolve 1 g NED in about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. Transfer to a glass or amber screw-cap container. This solution is stable for 6 months at 20°C.

7.6 Nitrate Reductase (NaR): follow the manufacturer's instructions for preparing a solution of 1 unit NaR (refer to section 3.1) activity per mL of phosphate buffer (refer to section 10.1). Dilute 3 units NaR to 20 mL with phosphate buffer. Store the solution at 2-6°C, where it is stable for 8 hours. Prepare sufficient NaR for the total number of samples and standards to be analyzed. **NOTE:** For some NaR forms, high phenolic content humic substances (>2 mg dissolved organic carbon /L) have little effect on the NaR activity in the temperature range of 5-15°C, but become increasingly inhibitory in the temperature range of 20-40°C (Patton and Kryskalla, 2011; 2013) . Humic substances at the operation temperatures specified in this test method do not inhibit other forms of NaR, such as recombinant AtNaR2 (Campbell et al., 2006). If humic acids are expected to be present, the user must verify reduction efficiency of the NaR in use by analysis of Quality Control checks that approximate the sample matrix.

7.7 Nitrate Solution, Stock (1000 mg/L NO3-N): Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 h. Dissolve 7.218 g in water in about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. This solution is stable for up to 2 months with refrigeration. Alternatively, certified nitrate stock solutions are commercially available through chemical supply vendors and may be used.

7.8 Nitrate Solution, Standard (10 mg/L NO3-N): Dilute 10 mL of stock nitrate solution (7.7) to 1 L with Deionized water and store in a dark bottle. Prepare fresh as needed.

7.9 Nitrite Solution, Stock (1000mg/L NO2-N): Place about 7 g of potassium nitrite (KNO_2) in a tared 125-mL beaker and dry for about 24 h to a constant weight in a desiccator containing a suitable desiccant. Adjust the weight of the dry potassium nitrite to 6.072 g. Add 50 mL of water to the beaker, stir until dissolved, and transfer quantitatively to a 1000-mL volumetric flask. Dilute to the mark with deionized water and store in a sterilized bottle under refrigeration. Prepare fresh as needed. Alternatively, certified nitrite stock solutions are commercially available through chemical supply vendors and may be used. **NOTE**: Potassium nitrite is easily oxidized; use only dry, free flowing white, or yellowish white crystalline powder of this reagent.

7.10 Nitrite Solution, Standard (10 mg/L NO2-N): Dilute 10 mL of stock nitrite solution (7.9) to 1 L with water. This solution is unstable; prepare fresh as needed.

8. Sample Collection, Preservation, and Storage

8.1 Sample preservation and holding time requirements for drinking water samples are as follows:

8.1.1 For nitrate: Chill the sample to ≤4°C and analyze within 48 hours. If the sample is chlorinated, the holding time for an unacidified sample kept at 4°C is extended to 14 days.

8.1.2 For nitrite: Do not add acid. Chill the sample to ≤4°C and analyze within 48 hours of collection.

8.1.3 For nitrate-nitrite: Acidify to pH<2 with H_2SO_4 at the time of collection, and analyze within 28 days of collection.

8.2 Sample preservation and holding time requirements for wastewater samples or other samples to be used for compliance monitoring under the Clean Water Act.

8.2.1 For nitrate: Chill the sample to ≤6 C and analyze within 48 hours of collection.

8.2.2 For nitrite: Chill the sample to ≤6 C and analyze within 48 hours of collection.

8.2.3 For nitrate-nitrite: Acidify to pH <2 with H_2SO_4 at the time of collection, and analyze within 28 days of collection.

8.3. In all cases, samples should be analyzed as soon as possible after collection.

9. Quality Control (QC)

9.1 **Requirement for QC Analysis:** To be certain that analytical values obtained using the test method are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when analyzing nitrite-nitrate in drinking water.

9.2 **Initial Demonstration of Performance (IDP):** IDP is used to characterize instrument performance and laboratory performance prior to performing analyses by this test method and periodically as specified below. The laboratory must complete the IDP tests and demonstrate compliance with the acceptance standards before beginning to analyze samples.

9.2.1 **Instrument Calibration Range (ICR):** ICR must be determined initially and verified every six months or whenever a significant change in the instrument response is expected or observed. The initial demonstration of ICR must employ a number of standards sufficient to insure that the results are reproducible and statistically acceptable. Verification of ICR must use a minimum of a blank and three standards. If any verification data exceeds the nominal value of the standard by ±10%, ICR must be

reestablished. ICR data must be fitted with a statistics program using either linear or non-linear regression analysis and an equation relating instrument response to the nominal values of the standards for the calibration obtained. The regression coefficient (R^2) must be 0.999 or greater; if it is not, determine the problem and recalibrate the instrument.

9.2.2 **Quality Control Sample (QCS):** QCS is a standard of known concentration from an external source and different from calibration standards. QCS must be analyzed initially and quarterly or as required to meet QC requirements. If the QCS analysis result is not within ±10% of the known concentration, the test has failed. The source of the problem must be identified and corrected before continuing with IDP.

9.2.3 **Reduction Efficiency (RE):** Analyze a nitrite standard (Section 7.10) for determining the Reduction Efficiency. The nitrite standard will contain 2.5 mg Nitrite-N/L (or other mid-range concentration) and the absorbance at 540 nm (or appropriate measure of the instrument response) is compared to the absorbance at 540 nm (or appropriate measure of the instrument response) of a Certified Nitrate-N Standard of the same concentration as the Nitrite-N standard:

RE = [(Nitrate-N Standard A-540)/(Nitrite-N Standard A-540) X 100].

For acceptable results, RE must be 90% or greater. If the RE is unacceptable, the source(s) of the problem must be determined and the DA re-calibrated.
NOTE: The likely source of a problem with the RE is - either the NaR is bad and needs to be replaced, or the NADH has expired and needs to be replaced.

9.2.4 **Method Detection Limit (MDL):** The MDL must be established for the analyte using reagent water (blank) fortified at a concentration near the detection limit of the instrument. Perform the MDL study according to "40 CFR Part 136 Appendix B". Samples should be prepared using reagent water fortified with nitrate at a concentration between 1 to 5 times the expected MDL. First, calibrate the instrument (see section 9.3). MDL is determined by analyses of seven replicates of fortified reagent water (spiked) and processed through the entire analytical method. Calculate the replicates analyte concentration in system units using the instrument calibration (see Section 10). Calculate MDL as follows:

$$MDL = (t) \times (S)$$

Where,

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.143 for seven replicates) and

S = standard deviation of the seven replicate analyses.

If the concentration of the sample (spike) used to determine the MDL is greater than 5X the calculated MDL repeat the MDL determination at a lower concentration for the sample (spike) studied. MDL should be determined every six months, when a new

operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 **Calibration and Calibration Verification:** Prior to the analysis of samples, calibrate the instrument using at least four working standards containing concentrations of nitrate in reagent water that bracket the expected sample concentration. Before each analysis, verify the calibration with a mid-range second source calibrant (SSC) from an external source and different from calibration standards. The result should fall within ± 10% of the known concentration. If the SSC is not within ± 10%, determine the problem, and recalibrate the instrument.

9.4 **Initial Performance and Recovery (IPR):** If a laboratory has not performed this test method before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analyze four replicates of a standard solution prepared from a Certified Independent Reference Material (CRM) containing a mid-range concentration of nitrite-nitrate in water. The matrix and chemistry of the solution should be equivalent to the solution used in the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps. Calculate the mean and standard deviation of the four values and compare to the acceptable ranges provided by the manufacturer of the CRM. Do not use this test method to analyze samples unless the IPR recoveries are within the accepted limits. Acceptable limits are ±10% of the known value.

9.5 **Ongoing Performance and Recovery Sample (OPR):** To ensure that the test method is in control, analyze a OPR containing a mid-range concentration of nitrite-nitrate in water with each batch or 10 samples. If large numbers of samples are analyzed in the batch, analyze the OPR after every 10 samples. The OPR must be taken through all of the steps of the analytical method including sample preservation and pretreatment. The result obtained for the OPR shall fall within ± 10% of the known concentration. If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all the samples in the batch must be reanalyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.6 **Method Blank:** Analyze a reagent water test blank with each batch or 10 samples. The concentration of nitrite-nitrate in water found in the blank should be less than the MDL. If the concentration of nitrite-nitrate in water is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.7 **Matrix Spike (MS) and Matrix Spike Duplicate (MSD):** To check for interferences in the specific matrix being tested, perform MS and MSD on at least one sample from each batch by spiking an aliquot of the sample with a known concentration of nitrite-nitrate in water and taking it through the analytical method. The spike concentration

plus the background concentration of nitrite-nitrate in water must not exceed the highest calibration standard. The spike must produce a concentration in the spiked sample that is 2 to 5 times the nitrite-nitrate in water concentration in the unspiked sample, or 10 to 50 times the detection limit of the test method, whichever is greater.

Calculate the percent recovery of the spike (P) using the following formula:

$$P = 100 \times \frac{[A \times (V_s + V) - (B \times V_s)]}{C \times V}$$

In this equation, A = nitrite+nitrate concentration (mg/L) in spiked samples, B = nitrite+nitrate concentration (mg/L) in unspiked samples, C = nitrite+nitrate concentration (mg/L) in the spiking solution, V_s = sample volume (mL) used, and V = added spiking solution volume (mL).

The percent recovery of the spike shall fall within ±10%, based on the nitrite-nitrate concentration in the spike. If the percent recovery is not within these limits, a matrix interference may be present in the sample selected for spiking. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.8 **Independent Reference Material (IRM):** In order to verify the quantitative value produced by the test method, analyze an Independent Reference Material (IRM) submitted as a regular sample (if practical) to the laboratory at least once per quarter. The concentration of the IRM should be in the concentration mid-range for the method chosen. The value obtained must fall within the control limits established by the laboratory.

10. Calibration and Standardization

10.1 Using the standard nitrate solution (section 7.8) prepare calibration standards by using the automated calibration function of the discrete analyzer (section 3.2). Table 1 specifies suggested calibrants. **NOTE**: Most discrete analyzers generate calibration standards and calibration curves automatically using Certified calibrants. Follow the manufacturer's instructions for calibrating with individual calibration standards, if an automatic calibration function is not available.

10.2 Prepare at least one calibration standard from the standard nitrite solution (section 7.10) at the same concentration as one of the nitrate standards to verify the efficiency of the reduction. Verify that reduction efficiency is greater than 90% with each batch of enzyme.

10.3 Run the calibration analysis as described in section 11 for sample analysis.

10.4 Prepare a standard curve by plotting the absorbance of each processed calibration standard against its known concentration. **NOTE**: Most discrete analyzers generate calibration curves automatically.

11. Procedure

11.1 Removal of Color Interferences: If there is a possibility that the color of the sample might absorb in the photometric range from 540 ± 20 nm, determine the background absorbance. **NOTE**: Many discrete analyzers automatically compensate for background absorbance and turbidity on each sample. Follow the manufacturer's instructions.

11.2 Prepare a method in the discrete analyzer software following these specifications:

- 11.2.1 Dispense 55 µL of NaR (section 7.6) and 5 µL of sample. Mix.
  NOTE: Larger volumes of samples and reagents may be used while maintaining the same ratio (See Table 2).
- 11.2.2 Add 12 µL of NADH (section 7.3). Mix and measure the background absorbance.
- 11.2.3 Incubate 600 seconds at 37ºC.
- 11.2.4 Add 25 µL of SAN reagent (section 7.4). Mix and incubate 120 seconds at 37ºC.
- 11.2.5 Add 25 µL of NED reagent (section 7.5). Mix and incubate 120 seconds at 37ºC.
- 11.2.6 Measure absorbance at 540 nm, using an optional background subtraction at 700 nm. If automatic background subtraction is not available, prepare a reagent blank (with SAN only added) for background subtraction at 540 nm.

11.3 When determining nitrite alone, replace NaR reagent (section 7.6) with Phosphate Buffer (section 7.1).

12. Data Analysis and Calculations

12.1 Determine the concentration of nitrate or nitrite nitrogen in the samples in mg N/L using the computer based data handler provided with the automated discrete analyzer software. **NOTE**: The discrete analyzer will automatically calculate the net absorbance by subtracting the background absorbance from the measured absorbance of the color developed sample. Use the net absorbance to determine the concentration of nitrogen in the sample.

12.2 Where separate values are required for nitrite-nitrogen and nitrate-nitrogen, calculate the nitrate-nitrogen by subtracting the nitrite-nitrogen from the total nitrate-nitrite nitrogen content.

12.3 Report the nitrogen content in mg N/L as:
Nitrite-Nitrogen (NO_2-N), mg N/L;
Nitrate-Nitrogen (NO_3-N), mg N/L; and
Combined Nitrate-Nitrite Nitrogen (NO_3, NO_2-N), mg N/L.

13. Method Performance

13.1 The performance of this method was validated by an Inter-Laboratory Study (See Plan for Inter-Laboratory Test Trial) and results of that study are delineated here.

13.2 Method Performance Data

13.2.1 Table 3. Drinking Water Matrices for Analysis

13.2.2 Table 4. Calibration of Enzymatic and Cadmium Reduction Methods Summary

13.2.3 Table 5. Enzymatic and Cadmium Reduction Efficiency Summary

13.2.4 Table 6. Initial Performance and Recovery (IPR) Summary and Ongoing Performance and Recovery (OPR) Summary

13.2.5 Table 7. Minimum Detection Limit (MDL) Summary

13.2.6 Table 8. DW-1 and DW-1-Cl Tap Water Matrix Nitrate-N Content and Spike Analysis

13.2.7 Table 9. DW-2 High TDS Drinking Water Matrix Nitrate-N Content and Spike Analysis

13.2.8 Table 10. DW-3 High TOC Drinking Water Matrix Nitrate-N Content and Spike Analysis

13.2.9 Table 11. Reference Standard RS-1 Summary

14. Pollution Prevention

All reagents and standards should be prepared in volumes consistent with laboratory use to minimize the generation of waste.

15. Waste Management

All waste should be disposed of in a manner consistent with local regulations. **NOTE**: This method uses very small volumes of samples and reagents and, therefore, less waste is generated than with most analysis methods.

16. References

Campbell, Wilbur H., P Song, GG Barbier (2006) Nitrate Reductase for Nitrate Analysis in Water. Environmental Chemistry Letters, 4: 69-73.

Patton, C.J., and Kryskalla, J.R., 2011, Colorimetric determination of nitrate plus nitrite in water by enzymatic reduction, automated discrete analyzer methods: U.S. Geological Survey Techniques and Methods, book 5, chap. B8, 34 p. (Available on line at http://pubs.usgs.gov/tm/05b08/).

Patton, C.J., and Kryskalla, J.R., 2013, Analytical properties of some commercially available nitrate reductase enzymes evaluated as replacements for cadmium in automated, semiautomated, and manual colorimetric methods for determination of nitrate plus nitrite in water: U.S. Geological Survey Scientific Investigations Report 2013–5033, 36 p., http://pubs.usgs.gov/sir/2013/5033/.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1993. Methods for Chemical Analysis of Water and Wastes. Method 353.2. Revision 2.0. U.S. Environmental Protection Agency, Washington, DC

17. Tables, Diagrams, Forms, Flowcharts, and Validation Data

TABLE 1 Example Concentrations of Calibration Standards

NO_3^--N or NO_2-N, mg/L	mL of 10 mg N/L Standard per 100 mL final volume
0.01	0.1
0.05	0.5
0.1	1.0
0.5	5.0
1.0	10
2.0	20
3.0	30
5.0	50

Table 2. Scaled sample and reagent volumes for implementation at larger assay volumes.

Volume (µL)	x1	x2	x3	x4	x5
Sample	5	10	15	20	25
AtNaR2	55	110	165	220	275
NADH	12	24	36	48	60
SAN	25	50	75	100	125
NED	25	50	75	100	125
Total Assay	122	244	366	488	610

Table 3

Drinking Water Matrices for Analysis

Each Sample Matrix (except DW-1) will be provided to Participating Laboratories as "Ready to Analyze" (samples will be filtered with 0.45 µm filter and preserved by acidifying) and in sufficient volume to permit multiple analysis. We will supply each lab with Chlorine Standard Solution, 25-30 mg/L as Cl_2, 2 mL PourRite Ampules (NIST)

Sample Matrix	Identifier	Number Replicates	Spikes*	Laboratories
Finished drinking water: from cold water tap of participating laboratories on day of analysis	DW-1	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	All
Local Tap Water with 2-4 mg/L free chlorine added	DW-1 + Cl_2	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	All
Drinking water with high TDS (~ 500 ppm)	DW-2	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	Lab 3
Drinking water with high TOC (~2 ppm)	DW-3	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	Lab 2
ERA #698 Standard Reference	SR-1**	7	NONE	All

*Each MS and MSD sample will be analyzed once. See Appendix D for method of preparing spikes and the layout of a set of analysis of typical DW-1 and its spikes.

SR-1 is Reference Standard of known Nitrate-N mg/L concentration (not acidified) and **must be analyzed by all Participating Laboratories. **SR-1 will require dilution with reagent water.**

Table 4

Calibrants and Standard Calibration Curve Equation for Participating Labs

	Lab 1		Lab 2		Lab 3	
Calibration	**NaR-R**	**Cd-R**	**NaR-R**	**Cd-R**	**NaR-R**	**Cd-R**
Reagent Blank	0.00	0.00	0.00	0.00	0.00	0.000
Calibrant 1	0.05	0.10	0.05	0.05	0.05	0.050
Calibrant 2	0.25	0.25	0.10	0.10	0.10	0.125
Calibrant 3	0.50	0.50	0.25	0.25	0.50	0.250
Calibrant 4	0.75	1.00	0.50	0.50	1.00	0.500
Calibrant 5	1.25	5.00	1.25	1.25	2.50	1.000
Calibrant 6	2.50	10.00	2.50	2.50	5.00	2.500
Calibrant 7	3.75	20.00	5.00	5.00		5.000
Calibrant 8	5.00					
Linear Regression	**Nitrate (mg N/L) = (Absorbance Sample - Intercept) / Slope**					
Slope	0.118	**Not Used in These Analyses**				
Intercept	0.022					
R^2	0.99989					
Polynomial Regression	**Nitrate (mg N/L) = (A x Conc x Conc) + (B x Conc) + C *for Lab 1* or = (A x A-550 x A-550) + (B x A-550) + C *for Lab 2* Or = (A x Area x Area) + (B x Area) + C *for Lab 3***					
Constant A	**---**	-0.029	-0.1988	0.4224	0.0018733	.00010392
Constant B	**---**	1.262	15.0219	5.1881	0.5099	0.0472
Constant C	**---**	0.018	-0.5308	-0.1879	0.1713	0.0011335
R^2	**---**	0.99996	1.00000	1.00000	0.99993	1.00000

Table 5

All Data from All Labs for

Enzymatic and Cadmium Reduction Efficiency

Evaluated using Certified Standards for 2.50 Nitrate-N mg/L and 2.50 Nitrite-N mg/L

Reduction Efficiency of greater than 90% is acceptance criterion.

	Lab 1		Lab 2		Lab 3*	
	NaR-R	**Cd-R**	**NaR-R**	**Cd-R**	**NaR-R**	**Cd-R**
Nitrate Absorbance 540 nm, 550 nm, Or Area/sec	0.318	0.495 0.491	0.2080 0.1982 0.1909 0.1929 0.1792	0.4915 0.4832 0.4420 0.4018 0.5390	5.4019 9.1622	47.942 28.501
Nitrite Absorbance 540 nm, 550 nm, Or Area/sec	0.341	0.527 0.501	0.1963 0.1892 0.1853 0.1881 0.1787	0.5082 0.4981 0.4484 0.4024 0.5777	5.7256 8.9972	49.528 27.381
Catalytic Reduction Efficiency (NO_3/NO_2) Percent	93.2551	93.9279 98.0040	105.9603 104.7569 103.0221 102.5518 100.2798	96.7139 97.0086 98.5727 99.8509 93.3010	94.3464 101.8339	96.7978 104.0904

*Lab 3 ran a series of 10-12 of these tests, which are not shown here.

Table 6

All Data Reported by Each Lab for

Initial Performance and Recovery (IPR)

and

Ongoing Performance and Recovery (OPR)

	Lab 1		Lab 2*		Lab 3	
	NaR-R	Cd-R	NaR-R	Cd-R	NaR-R	Cd-R
IPR Nitrate-N mg/L	2.01	2.01	2.01	2.01	2.01	2.01
Number IPR	4	8	16	16	8	8
Mean IPR	2.0705	1.9598	1.9682	1.9921	1.9951	1.9827
Mean IPR Recovery (%)	103.0075	97.5044	97.9201	99.1091	99.2562	98.6412
OPR Nitrate-N mg/L	2.01	12.00	2.50	2.50	2.50	2.50
Number OPR	7	8	15	16	14	13
Mean OPR	2.1388	11.8393	2.4769	2.5189	2.4877	2.5319
Standard Deviation of Mean	0.03685	0.208999	0.07204	0.1145	0.11536	0.01287
Relative Standard Deviation (%)	1.7233	1.7653	2.9085	4.5475	4.6372	0.5082
Mean Recovery (%)	106.4065	98.6610	99.0740	100.7564	99.5083	101.277

*Lab 2 Cd-R had 1 OPR greater than 110%

Table 7

Minimum Detection Limit

7 Replicates

MDL = Standard Deviation x 3.143

Abbreviations: DA, Discrete Analyzer; FIA, Flow Injection Analyzer

	Lab 1		Lab 2		Lab 3	
	NaR-R	Cd-R	NaR-R	Cd-R	NaR-R	Cd-R
Analytical Equipment	**DA**	**DA**	**DA**	**DA**	**FIA**	**FIA**
Spike mg Nitrate-N/L	**0.050**	**0.050**	**0.025**	**0.020**	**0.010**	**0.010**
MDL mg Nitrate-N/L	**0.0097**	**0.03436**	**0.006507**	**0.006694**	**0.009301**	**0.004721**
Ratio Spike/MDL	5.1453	1.4553	3.8422	2.9877	1.0751	2.1182

Table 8. A. DW-1 and DW-1-Cl - Lab 1 Tap Water Matrix Nitrate-N Content and Spike Analysis

Lab 1 NaR Reduction Method DW1 (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

A-540	Nitrate-N mg/L	Mean ± Standard Deviation Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.059	0.3207	0.3237 ± 0.003698 RSD 1.1424%	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.059	0.3215		0.179	1.3392	0.4859	97.1829	0.9830	0.179	1.3392	1.0155	101.5514	0.3947	0.366	2.9467	2.6230	104.9206	0.4451
0.06	0.3282																
0.06	0.3279		0.178	1.3352	0.4907	98.1429		0.178	1.3352	1.0115	101.1514		0.367	2.9584	2.6347	105.3886	
0.06	0.3235																
0.06	0.3255																
0.059	0.3185																

Lab 1 NaR Reduction Method DW1-Cl

A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.058	0.3117	0.3185 ± 0.01625 RSD 5.1020%	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.058	0.3122		0.118	0.8191	0.5006	100.1200	1.4082	0.175	1.3087	0.9902	99.0200	1.6923	0.367	2.9515	2.6330	105.3200	0.3564
0.063	0.3547																
0.059	0.3175		0.117	0.8121	0.4936	98.7200		0.177	1.3256	1.0071	100.7100		0.368	2.9609	2.6424	105.6960	
0.059	0.3136																
0.058	0.3126																
0.058	0.3072																

Lab 1 Cd Reduction Method DW1

A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.066	0.2429	0.2259 ± 0.008633 RSD 3.7773%	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.063	0.2260		0.154	0.7066	0.4807	96.1486	7.1602	0.258	1.2516	1.0257	102.5743	2.1581	0.526	2.6585	2.4326	97.3057	0.7331
0.062	0.2217																
0.064	0.2305		0.161	0.7423	0.5164	103.2886		0.253	1.2297	1.0038	100.3843		0.529	2.6764	2.4505	98.0217	
0.062	0.2230																
0.062	0.2185																
0.062	0.2184																

Lab 1 Cd Reduction Method DW1-Cl

A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.063	0.2233	0.2186 ± 0.007162 RSD 3.2763%	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.062	0.2224		0.159	0.7353	0.5167	103.3371	4.0278	0.258	1.2515	1.0329	103.2886	2.6884	0.538	2.7229	2.5043	100.1714	3.4193
0.063	0.2267																
0.061	0.2173		0.156	0.7149	0.4963	99.2571		0.252	1.2241	1.0055	100.5486		0.532	2.6387	2.4201	96.8038	
0.061	0.2151																
0.062	0.2206																
0.059	0.2049																

Table 8. B. DW-1 and DW-1-Cl - Lab 2 Data Set 2 Tap Water Matrix Nitrate-N Content and Spike Analysis

Lab 2 NaR Reduction Method DW1 (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

A-550 nm	Nitrate-N mg/L	Mean ± Standard Deviation Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0825	0.9304	0.94569 ± 0.016307 RSD 1.7243%															
0.0835	0.9464		0.1151	1.4501	0.5044	100.8837	3.5318	0.1437	1.9045	0.9588	95.8841	4.3690	0.2315	3.2907	2.3451	93.8021	2.4482
0.0828	0.9352																
0.0852	0.9735		0.1140	1.4326	0.4869	97.3825		0.1464	1.9474	1.0017	100.1668		0.2352	3.3489	2.4032	96.1270	
0.0838	0.9512																
0.0823	0.9272																
0.0841	0.9560																

Lab 2 NaR Reduction Method DW1-Cl

A-550 nm	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0820	0.9224	0.8546 ± 0.03249 RSD 3.8017%															
0.0775	0.8505		0.1091	1.3546	0.5000	99.9911	3.8968	0.1361	1.7839	0.9293	92.9273	0.8506	0.2241	3.1744	2.3198	92.7913	2.0549
0.0770	0.8425																
0.0773	0.8473		0.0562	0.4791	0.4808	96.1691		0.1366	1.7919	0.9372	93.7211		0.1880	2.3527	2.2726	90.9040	
0.0764	0.8330																
0.0783	0.8633																
0.0758	0.8234																

Lab 2 Cd Reduction Method DW1

A-550 nm	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.2295	0.9208	0.910579 ± 0.025085 RSD 2.7549%	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %
0.2195	0.8726		0.3248	1.3804	0.4699	93.9708	1.4507	0.4188	1.8358	0.9252	92.5184	0.2101	0.7264	3.3391	2.4285	97.1403	0.7933
0.2246	0.8972																
0.2235	0.8919		0.3234	1.3737	0.4631	92.6174		0.4184	1.8338	0.9232	92.3242		0.7225	3.3199	2.4093	96.3728	
0.2327	0.9362																
0.2278	0.9126																
0.2341	0.9429																

Lab 2 Cd Reduction Method DW1-Cl

A-550 nm	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.2256	0.9020	0.9050 ± 0.009564 RSD 1.0567%	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %
0.2278	0.9126		0.3236	1.3746	0.4696	93.9249	6.1848	0.4213	1.8479	0.9429	94.2891	1.4314	0.7270	3.3420	2.4370	97.4812	1.6897
0.2250	0.8991																
0.2237	0.8928		0.3298	1.4046	0.4996	99.9193		0.4241	1.8615	0.9565	95.6484		0.7187	3.3012	2.3962	95.8479	
0.2289	0.9179																
0.2246	0.8972																
0.2280	0.9135																

Table 8. C. DW-1 and DW-1-Cl - Lab 3 Tap Water Matrix Nitrate-N Content and Spike Analysis

(Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

Lab 3 NaR Reduction Method DW1 Data Set 30Oct2015

Area V/s 540 nm	Nitrate-N mg/L	Mean ± Standard Deviation Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.53691	0.03439	0.03438 ± 0.0006398 RSD 1.8608%	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.52026	0.03541		1.4411	0.5674	0.5674	106.6035	2.5541	2.5271	1.1292	1.094818	109.4818	0.3283	5.2217	2.5423	2.507918	100.3167	1.9232
0.53262	0.03376																
0.53577	0.03385		1.4678	0.58112	0.5468	109.3615		2.5341	1.1328	1.098418	109.8418		0.367	2.9584	2.6347	105.3886	
0.52710	0.03393																
0.52747	0.03426																
0.53226	0.03509																

Lab 3 NaR Reduction Method DW1-Cl Data Set 30Oct2015

Area V/s A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.5681	0.1190	0.1166 ± 0.004657 RSD 3.9943%	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.5703	0.1201		1.4923	0.5938	0.4772	95.44171	0.05210	2.4589	1.0938	0.9772	97.7208	0.05118	5.2544	2.5597	2.4431	97.7243	0.2213
0.5688	0.1194																
0.5627	0.1185		1.4875	0.5913	0.4747	94.94571		2.4578	1.0933	0.9767	97.6709		5.2558	2.5543	2.4377	97.5083	
0.5471	0.1082																
0.5539	0.1117																
0.5685	0.1192																

Lab 3 Cd Reduction Method DW1 Data Set 30Oct2015

Area V/s A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
1.7679	0.0849	0.08778 ± 0.001313 RSD 1.4962%															
1.8455	0.0886		11.749	0.5700	0.4822	96.4493	0.1326	21.883	1.0838	0.9960	99.6017	0.6405	50.04	2.6233	2.5355	101.4207	0.0316
1.8438	0.0885																
1.8407	0.0884		11.762	0.5707	0.4829	96.5773		21.983	1.0902	1.0024	100.2417		50.026	2.6225	2.5347	101.3887	
1.8407	0.0884																
1.8363	0.0882																
1.8240	0.0876																

Lab 3 Cd Reduction Method DW1-Cl Data Set 30Oct2015

Area V/s A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
2.8934	0.1386	0.1387 ± 0.0004249 RSD 0.3063%															
2.9201	0.1390		11.616	0.5634	0.4294	85.8743	0.1350	21.738	1.0763	0.9376	93.7571	0.2135	49.520	2.5933	2.4546	98.1829	0.2978
2.9069	0.1392																
2.9061	0.1392		11.722	0.5687	0.4300	85.9903		21.699	1.0743	0.9356	93.5571		49.392	2.5860	2.4473	97.8909	
2.8956	0.1387																
2.8895	0.1384																
2.8831	0.1381																

Table 9

DW-2 High TDS Drinking Water Matrix Nitrate-N Content and Spike Analysis

Abbreviation: RPD = Relative Percent Difference

Lab 3 NaR Reduction Method DW-2

Nitrate-N mg/L	Mean Nitrate-N mg/L	Standard Deviation mg N/L	Relative Standard Deviation
0.2907	0.2959	0.004749	1.6048%
0.2973			
0.3023			
0.2985			
0.2939			
0.2992			
0.2893			

0.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
1.944	0.7900	0.4941	98.8226	0.0486
1.9437	0.7898	0.4939	98.7746	

1.0 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
2.8302	1.2489	0.9530	95.3003	0.3875
2.8375	1.2526	0.9567	95.6703	

2.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
5.8573	2.8417	2.5458	101.8321	0.4251
5.8371	2.8309	2.5350	101.4001	

Lab 3 Cd Reduction Method DW-2

Nitrate-N mg/L	Mean Nitrate-N mg/L	Standard Deviation mg N/L	Relative Standard Deviation
0.2659	0.2677	0.001071	0.4000%
0.2684			
0.2678			
0.2679			
0.2680			
0.2693			
0.2669			

0.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
15.418	0.7536	0.4858	97.1697	0.4125
15.386	0.7516	0.4838	96.7697	

1.0 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
24.932	1.2425	0.9748	97.4759	0.2050
24.970	1.2445	0.9768	97.6759	

2.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
53.061	2.7602	2.4925	99.6983	0.5512
52.407	2.7465	2.4788	99.1503	

Table 10

DW-3 High TOC Drinking Water Matrix Nitrate-N Content and Spike Analysis

Abbreviation: RPD = Relative Percent Difference

Lab 2 NaR Reduction Method DW-3

Nitrate-N mg/L	Mean Nitrate-N mg/L	Standard Deviation mg N/L	Relative Standard Deviation
-0.0213	-0.020617	0.001497	7.2622%
-0.0228			
-0.0199			
-0.0199			
-0.0185			
-0.0213			
-0.0143			

0.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0507	0.4212	0.4418	88.3633	5.5884
0.0525	0.4466	0.4672	93.4433	

1.0 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0870	0.9320	0.9526	95.2617	1.0341
0.0863	0.9222	0.9428	94.2817	

2.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.1878	2.3364	2.3570	94.2807	0.7068
0.1866	2.3198	2.3404	93.6167	

Lab 2 Cd Reduction Method DW-3

Nitrate-N mg/L	Mean Nitrate-N mg/L	Standard Deviation mg N/L	Relative Standard Deviation
0.0044	0.002450	0.002696	110.0301%
0.0018			
0.0057			
-0.0021			
0.0031			
0.0018			
0.0025			

0.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.1106	0.5147	0.5123	102.4500	3.0723
0.1082	0.4992	0.4968	99.3500	

1.0 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.1923	1.0417	1.0393	103.9250	1.5373
0.1948	1.0578	1.0554	105.5350	

2.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.4405	2.6422	2.6398	105.5900	3.2766
0.4273	2.5571	2.5547	102.1860	

Table 11

Standard Reference - SR-1 (Seven Replicates Analyzed)

ERA 698 Certified Value **SR-1 = 5.14 ± 1.83% mg Nitrate-N/L**

	Dilution	Diluted Mean mg Nitrate-N/L	Standard Deviation mg Nitrate-N/L	Relative Standard Deviation %	Final mg Nitrate-N/L	Compared to ERA
Lab 1 - NaRR	Auto-diluted 1:10	5.3989	0.03375	0.6251	5.3989	105.0195%
Lab 1 - CdR	NONE	5.0937	0.07998	1.5701	5.0937	99.0992%
Lab 2 - NaRR	Auto-diluted 1:4	4.9795	0.07552	1.5166	4.9795	96.8783%
Lab 2 - CdR	1:4	1.0128	0.02132	2.1052	5.0640	98.5214%
Lab 3 - NaRR	1:2	2.5629	0.005100	0.1990	5.1258	99.7218%
Lab 3 - CdR	1:2	2.5902	0.01816	0.7011	5.1800	100.7782%