LABORATORY NAME:		CERT #:	
PRIMARY ANALYST:		DATE:	
NAME OF PERSON COMPLETING CHECKLIST (PRINT):			
SIGNATURE OF PERSON COMPLETING CHECKLIST:			

Parameter: **Nitrogen, Nitrate + Nitrite**

Method: **SM 4500 NO_3^- F- 2019**

Note: This is an automated version of SM 4500 NO_3^- E- 2019. As the verbiage in SM 4500 NO_3^- F- 2019 is sparse, many of the method references contained in this checklist will refer to the manual method.

Equipment:

	Automated analytical instrument
	Reduction column
	0.45-µm membrane filters (if applicable)

Reagents:

	Cadmium granules		Ammonium chloride-EDTA solution		Copper sulfate solution, 2%
	Color reagent		Hydrochloric acid, 6M		Stock nitrate solution
	Wash solution (reagent water)		Sulfuric Acid, concentrated		Stock nitrite solution

PLEASE COMPLETE CHECKLIST IN INDELIBLE INK
Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

	GENERAL	LAB	SOP	EXPLANATION
1	Is the SOP reviewed at least every 2 years? What is the most recent review/revision date of the SOP? [15A NCAC 02H .0805 (a) (7)] **Date:**			Quality assurance, quality control, and Standard Operating Procedure documentation shall indicate the effective date of the document and be reviewed every two years and updated if changes in procedures are made. Verify proper method reference. During review notate deviations from the approved method and SOP.
2	Are all review/revision dates and procedural edits tracked and documented? [15A NCAC 02H .0805 (a) (7)]			Each laboratory shall have a formal process to track and document review dates and any revisions made in all quality assurance, quality control and SOP documents.
3	Is there North Carolina data available for review?			If not, review PT data
	PRESERVATION and STORAGE	**LAB**	**SOP**	**EXPLANATION**
4	Is the sample preserved with H_2SO_4 to pH <2 S.U. within 15 minutes of collection? [40 CFR Part 136.3, Table II and footnote 2]			
5	Is sample transported and stored at ≤ 6°C without freezing? [40 CFR Part 136.3, Table II and footnote 18]			
6	Is the sample analyzed within 28 days of collection? [40 CFR Part 136.3, Table II]			
7	Are date and time of sample collection documented? [15A NCAC 02H .0805 (a) (7) (F) (vi)]			
8	Is the date of sample analysis documented? [15A NCAC 02H .0805 (a) (7) (F) (vii)]			
	PROCEDURE – Reduction Column Preparation	**LAB**	**SOP**	**EXPLANATION**
9	Is the reduction column purchased already packed? **If yes, skip to next section for Meter Calibration**			
10	Are the cadmium granules prepared as required by the method? [SM 4500 NO_3^- E- 2019 (3) (b)]			Wash 25 g new or used 20- to 100-mesh Cd granules with 6N HCl and rinse with water. Swirl Cd with 100 mL 2% $CuSO_4$ solution for 5 min or until blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to develop. Gently flush with ammonium chloride-EDTA solution to remove all precipitated Cu.

11	Is the column prepared as required by the method? [SM 4500 NO_3^- F-2019 (3) (d)]			Prepare the cadmium-reduction column according to the manufacturer's instructions or purchase a premade cadmium-reduction column or coil from the instrument manufacturer.
	PROCEDURE – Instrument Calibration	**LAB**	**SOP**	**EXPLANATION**
12	Is the instrument calibrated with at least 5 non-zero standards? [SM 4500 NO_3^- E- 2019 (4) (c)] **List standard concentrations:**			The method requires 5 standards, so curves prepared daily must still analyze 5 standards.
13	If the curve is held, is it prepared every 12 months? [15A NCAC 02H .0805 (a) (7) (H) (v)]			
	PROCEDURE – Interferences	**LAB**	**SOP**	**EXPLANATION**
14	Is the sample filtered if turbid? [SM 4500 NO_3^- F-2019 (1) (b)]			Suspended matter may restrict sample flow in the column, so filter turbid samples (see 4500-NO_3^- A.1). Filter turbid sample through 0.45-µm membrane filter. Test filters for nitrate contamination. (i.e., filter the reagent blank if any samples must be filtered)
15	Are aliquots of samples that are treated for residual chlorine in the field brought to a neutral pH and verified to be chlorine free when received in the lab? [SM 4500 NO_3^- F-2019 (1) (b)] [NC WW/GW LCB Sample Collection, Preservation, Storage and Transport Requirements for Non-Field Laboratories Policy]			NC WW/GW LCB Policy: Each chemically preserved sample must be checked for effectiveness and the results documented. Dechlorinating agents used at the time of sampling must be documented to have been effective (either by the sample collector or the receiving laboratory) by verifying a chlorine residual <0.5 mg/L at a neutral pH. When measuring chlorine concentration in an acidified sample, pour off a small portion of the sample and neutralize the pH prior to testing. Use sufficiently strong base to not dilute the sample. Discard that portion after testing.
	PROCEDURE – Sample Analysis	**LAB**	**SOP**	**EXPLANATION**
16	Is the sample pH adjusted to 5-9 S.U.? [SM 4500 NO_3^- F-2019 (4)]			
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
17	Has a Method Detection Limit (MDL) been established? [SM 4500 NO_3^- A-2019 (3)] [40 CFR 136 Appendix B] **State MDL value here:** **State determination date here:**			The initial MDL determination must consist of minimum of 7 spikes and 7 method blanks. They must be divided among 3 separate prep batches on 3 separate days.
18	Are at least two spikes at the same concentration as the initial MDL study analyzed in separate batches each quarter that samples are analyzed? [40 CFR 136 Appendix B]			Must have at least two per quarter, however if additional standard at that concentration are analyzed, they must be included in the ongoing recalculation of the MDL.
19	Is the MDL evaluated at least every 13 months and updated if required? [40 CFR 136 Appendix B]			
20	Has each new analyst completed an Initial Demonstration of Capability (IDC) before analyzing any samples? [SM 4500 NO_3^- A-2019 (3)] [SM 4020 B-2022 (3)] **Attach a copy of each analyst's IDC to this checklist**.			At a minimum, include 1 reagent blank and at least 4 LFBs at a concentration between 1 and 4 times the MRL (or other level specified in the method). Run the IDC after analyzing all required calibration. To establish laboratory-generated accuracy and precision limits, calculate the upper and lower control limits from the mean and standard deviation of percent recovery for ≥20 data points: Upper control limit = Mean + 3(Standard deviation) Lower control limit = Mean − 3(Standard deviation)

21	Is the correlation coefficient of the calibration curve ≥0.995? [SM 4500 NO_3^- A-2019 (3)]			Using a calculator, electronic spreadsheet, or instrument software, calculate the slope, intercept, and correlation coefficient (r) or coefficient of determination (r^2) of the calibration curve. The r value must be at least 0.995 (r^2 = 0.99).
22	Are the standard values back-calculated with each calibration? [SM 4500 NO_3^- A-2019 (3)] [15A NCAC 02H .0805 (a) (7) (H)]			Back-calculate the apparent concentrations of the standards.
23	What are the acceptance criteria for the back-calculated standards? [SM 4020 B-2022 (1)] [SM 4500 NO_3^- A-2019 (3)] **Acceptance criteria:**			**4020B:** If any recalculated values are not within the method's acceptance criteria - up to twice the MRL, ±50%; between 3 and 5 times the MRL, ±20%; or greater than 5 times the MRL ±10%- unless otherwise specified in the individual methods, identify the source of any outlier(s) and correct before sample quantitation. **4500 NO_3^- A:** For standards more than 10 times the MDL, the measured values must be 90% to 110% of the true values.
24	Is a second-source calibration-verification standard (CVS) analyzed immediately after the calibration? [SM 4500 NO_3^- A-2019 (3)] [15A NCAC 2H .0805 (a) (7) (H) (ii)]			Prepare a calibration-verification standard (CVS) from a stock solution separate from that used to prepare the calibration standards. The CVS's NO_3^--N concentration should be 30% to 70% of the highest calibration standard; however, some QA/QC programs may require different concentrations. Run the CVS immediately after calibration; the result must be 90% to 110% of the expected value. **Rule:** Laboratories shall analyze one known second source standard to verify the accuracy of standard preparation if an initial calibration is performed and in accordance with the referenced method requirements thereafter.
25	Is the acceptance criterion for the second-source CVS recovery within ±10% of the true value? [SM 4500 NO_3^- A-2019 (3)] **True value:** **Acceptance criterion:**			See above
26	Is a standard at the lowest reporting concentration analyzed or back calculated each day samples are analyzed? [15A NCAC 02H .0805 (a) (7) (H)]			If the reporting limit is the same as one of the calibration standards, this will be covered by the curve back-calculation requirement, when the calibration curve is performed daily.
27	What is the acceptance criterion for the lowest reporting concentration standard? [SM 4020 B-2022 (1)] [SM 4500 NO_3^- A-2019 (3)] **Acceptance criterion:**			**4500 NO_3^- A:** For standards more than 10 times the MDL, the measured values must be 90% to 110% of the true values. **4020B:** If less than 10 times the MDL: For standards up to twice MRL, ±50%
28	Is a Laboratory Fortified Blank (LFB) analyzed with each sample set or on a 5% basis, whichever is more frequent? [SM 4020 B-2022 (6)]			
29	Is the LFB filtered if any samples require filtration? [SM 4020 B-2022 (6)]			Process the LFB through all sample preparation and analysis steps.
30	Is Sodium thiosulfate added to the LFB if any samples must be treated for residual chlorine? [SM 4020 B-2022 (6)]			
31	What is the acceptance criterion for the LFB? [SM 4020 B-2022 (6)] **Acceptance criterion:**			Evaluate the LFB for percent recovery of the added analytes by comparing results to method-specified limits, control charts, or other approved criteria. If there are a mix of both filtered and unfiltered samples, you must have both a filtered and unfiltered LFB.
32	Is a method blank analyzed with each sample set (batch) or on a 5% basis, whichever is more frequent? [SM 4020 B-2022 (5)]			
33	Is the method blank filtered if any samples require filtration? [SM 4020 B-2022 (5)]			If there is a mix of filtered and unfiltered samples, you must have both a filtered and unfiltered method blank.

34	Is Sodium thiosulfate added to the method blank if any samples must be treated for residual chlorine? [SM 4020 B-2022 (5)]			
35	Is the acceptance criterion for the method blank ≤½ reporting limit? [15A NCAC 02H .0805 (a) (7) (H) (i)]			
36	Is a midpoint continuing calibration verification (CCV) analyzed prior to sample analysis, after every 10th sample, and at the end of each sample group? [15A NCAC 02H .0805 (a) (7) (H)] [SM 4500 NO_3^- A-2019 (3)] **True Value:**			
37	Is the acceptance criterion for the CCV recovery within ±10% of the true value? [SM 4500 NO_3^- A-2019 (3)]			If the measured NO_3^- - N concentration in the CCV is not 90 to 110% of the expected value, recalibrate and rerun all samples read since the last good CCV reading.
38	Is a calibration blank analyzed prior to sample analysis, after every 10th sample, and at the end of each sample group? [15A NCAC 02H .0805 (a) (7) (H)] [SM 4500 NO_3^- A-2019 (3)]			
39	Is the acceptance criterion for the calibration blank ≤ ½ reporting limit? [15A NCAC 02H .0805 (a) (7) (H) (i)]			
40	Is a matrix spike (MS) and matrix spike duplicate (MSD) pair analyzed with each sample set (batch of 20 samples or less) or on a 5% basis, whichever is more frequent? [SM 4020 B-2022 (7)]			
41	How is the MS/MSD prepared? [NC WW/GW LCB Matrix Spike Technical Assistance Policy] [SM 4020 B-2022 (7)] **Answer:**			See NC WW/GW LCB "Matrix Spiking Policy and Technical Assistance" document for volume and sample dilution requirements. **SM states:** Add a concentration that is at least 10 x MRL, less than or equal to the midpoint of the calibration curve, or method-specified level to the selected sample(s). The analyst should use the same concentration as for LFB (4020 B.6) to allow analysts to separate the matrix's effect from laboratory performance. Prepare LFM from the same reference source used for LFB. Make the addition such that sample background levels do not adversely affect recovery (preferably adjust LFM concentrations if the known sample is more than 5 times the background level). At a minimum, the spike must at least equal the background concentration, unless the method specifies otherwise. For example, if the sample contains the analyte of interest, then add approximately as much analyte to the LFM sample as the concentration found in the known sample.
42	What is the acceptance criterion for the accuracy (recovery) of the MS/MSD? [SM 4020 B-2022 (7)] **Answer:**			**SM states:** Evaluate LFM results for percent recovery; if they are not within control limits, then take corrective action to rectify the matrix effect, use another method, use the method of standard addition, or flag the data if reported. See method for specific LFM acceptance criteria until the laboratory develops statistically valid, laboratory-specific performance criteria. If the method does not provide limits, use the calculated preliminary limits from the IDC (4020 B.3). LFM control limits may be wider than for LFB or LCS, and batch acceptance generally is not contingent upon LFM results.
43	What corrective action does the laboratory take if the MS/MSD results are outside of established control limits for **accuracy**? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			
44	What is the acceptance criterion for the precision (RPD) of the duplicates? [15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			

45	What corrective action does the laboratory take if the LFM/LFMD results are outside of established control limits for **precision**? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			
46	Is the stock nitrite solution standardized prior to use if prepared in-house? [SM 4500 NO_2^- B-2021 (3) (e)]			Stock nitrite solution: Commercial reagent-grade $NaNO_2$ assays at less than 99%. Because NO_2^- is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine $NaNO_2$ content, add a known excess of standard 0.05 N $KMnO_4$ solution (see ¶ h below), discharge permanganate color with a known quantity of standard reductant, such as 0.025 M $Na_2C_2O_4$ or 0.05 M $Fe(NH_4)_2(SO_4)_2$, and back-titrate with standard permanganate solution.
47	What is the expiration date for purchased and prepared stock nitrite solutions? [NC WW/GW LCB Chemical, Reagent, Standard and Consumables Expiration Date Policy] **Answer:**			If the method does not specify an expiration date, chemicals, reagents and standards prepared in the laboratory for use with that method must be assigned an expiration date according to the laboratory's policy for doing so. Monitor materials for changes in appearance or consistency. Any changes may indicate potential contamination or degradation, and therefore, the item must not be used, even if the manufacturer's or laboratory's expiration date has not been exceeded. Laboratory-assigned expiration dates may be re-evaluated based on performance and recovery data and new expiration dates assigned at that time.
48	Are intermediate and working NO_2^- standards prepared daily? [SM 4500 NO_2^- B-2021 (3) (f) and (g)]			Recipes are at the end of the checklist
49	Is at least one mid-level NO_2^- standard compared to a NO_3^- standard at the same concentration to verify reduction column efficiency? [SM 4500 NO_3^- F-2019 (6)]			Run a mid-level NO_3^--N standard followed immediately by a NO_2^--N standard of the same concentration. Calculate reduction efficiency as follows: Efficiency = (NO_3^--N response ÷ NO_2^--N response) × 100.
50	What is the acceptance criterion for reduction efficiency? [SM 4500 NO_3^- F-2019 (6)] **Answer:**			The efficiency must be 90% to 110%.
51	Are Cu-Cd granules reactivated if the reduction efficiency falls below 90%? [SM 4500 NO_3^- F-2019 (6)]			If not, stop and correct the problem by either following the manufacturer's instructions or passing 6 M HCl through the column followed by rinsing with dilute ammonium chloride-EDTA solution. Prepare or, if it cannot be reactivated, purchase a new column according to 4500-NO_3 E.3 b and activate according to 4500-NO_3 E.4 a.
52	Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [15A NCAC 02H .0805 (a) (7) (B)]			Reported data associated with quality control failures, improper sample collection, holding time exceedances, or improper preservation shall be qualified as such.

Stock nitrite solution: Commercial reagent-grade $NaNO_2$ assays at less than 99%, Because NO_2^- is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine $NaNO_2$ content, add a known excess of standard 0.05N $KMnO_4$ solution, discharge permanganate color with a known quantity of standard reductant such as 0.025M $Na_2C_2O_4$ or 0.05M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and back-titrate with standard permanganate solution.

1. Preparation of stock solution – Dissolve 1.232 g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 µg N. Preserve with 1 mL $CHCl_3$ (chloroform).
2. Standardization of stock nitrite solution – Pipet, in order, 50.00 mL standard 0.05N $KMnO_4$, 5 mL conc H_2SO_4, and 50.00 mL stock NO_2^- solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while adding stock NO_2^- solution. Shake gently and warm to 70 to 80°C on a hot plate. Discharge permanganate color by adding sufficient

10-mL portions of standard 0.025M $Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.05N $KMnO_4$ to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below.

NOTE: If ferrous ammonium sulfate solution is substituted for $Na_2C_2O_4$, omit heating and extend reaction period between $KMnO_4$ and Fe^{2+} to 5 min before making final $KMnO_4$ titration.

3. Calculate NO_2^--N content of stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

Where: A = mg NO_2^- -N/mL in stock $NaNO_2$ solution.

B = total mL standard $KMnO_4$ used.

C = normality of standard $KMnO_4$.

D = total mL standard reductant added.

E = normality of standard reductant, and

F = mL stock $NaNO_2$ solution taken for titration.

Each 1.00 mL 0.05N $KMnO_4$ consumed by the $NaNO_2$ solution corresponds to 1725 µg $NaNO_2$ or 350 µg NO_2^--N.

Intermediate nitrite solution: Calculate the volume, G of stock NO_2^- solution required for the intermediate NO_2^- solution from G = 12.5/A. Dilute the volume G (approximately 50 mL) to 250 mL with water; 1.00 mL = 50.0 µg N. Prepare daily.

Standard nitrite solution: Dilute 10.00 mL intermediate NO_2^- solution to 1000 mL with water; 1.00 mL = 0.500 µg N. Prepare daily.

Standard potassium permanganate titrant, 0.05N: Dissolve 1.6 g $KMnO_4$ in 1 L distilled water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet supernate without stirring up any sediment. Standardize this solution frequently by the following procedure:

Weigh to the nearest 0.1 mg several 100- to 200-mg samples of anhydrous $Na_2C_2O_4$ into 400-mL beakers. To each beaker, in turn, add 100 mL distilled water and stir to dissolve. Add 10 mL 1 + 1 H_2SO_4 and heat rapidly to 90 to 95°C. Titrate rapidly with permanganate solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg will consume about 6 mL solution. Run a blank on distilled water and H_2SO_4.

$$\text{Normality of } KMnO_4 = \frac{\text{g } Na_2C_2O_4}{(A - B) \times 0.067}$$

Where: A = mL titrant for sample, and

B = mL titrant for blank.

Average the results of several titrations.

Additional Comments:

____

____

____

____

____

____

____

____

____

____

____

____

____

____

Inspector: ____Date:______________________________________