NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION

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

Parameter: **Nitrite Nitrogen**

Method: **SM 4500-NO_2^- B-2011 (Aqueous)**
Manual Spectrophotometric

EQUIPMENT:

	Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer. **Model:**		Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm **Model:**		Filtration apparatus, for use with 0.45-µm-pore-diam membrane filters.

ANALYSIS REAGENTS:

	Nitrite-free water		Ferrous Ammonium Sulfate, 0.05*M* (0.05*N*)
	Color reagent		Stock Nitrite Solution, 1.00 ml = 250 µg N
	Sodium oxalate, 0.025*M* (0.05*N*) [$Na_2C_2O_4$]		Standard Potassium Permanganate titrant, 0.05*N*
	Hydrochloric acid, 1*N*		Ammonium hydroxide, 1*N*

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 2H .0805 (a) (7)] **ANSWER:**			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 revision dates and actions tracked and documented? [15A NCAC 2H .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	Are samples iced to above freezing but ≤ 6 º C during shipment? [40 CFR 136.3 Table II and footnote 18]			40 CFR footnote 2 allows 15 minutes for sample preservation, including thermal. This means that if a sample is received in the lab within 15 minutes it is not required to be on ice. Document temperature downward trend for short transport samples.
5	Are samples refrigerated above freezing to 6°C during storage? [40 CFR 136.3 Table II and footnote 18]			
6	Are samples analyzed within 48 hours of collection? [40 CFR 136.3 Table II]			
	PROCEDURE –Calibration	**LAB**	**SOP**	**EXPLANATION**
7	Is a standard curve constructed by plotting absorbance of standards against NO_2^--N concentration? [SM 4500- NO_2^- B-2011 (5)]			Prepare a standard curve by plotting absorbance of standards against NO_2^--N concentration. Compute sample concentration directly from curve.

8	**List the values of standards used for the calibration:** [15A NCAC 2H .0805 (a) (7) (H) and (H) (v)]			For analytical procedures requiring analysis of a series of standards, the concentrations of these standards shall bracket the range of the sample concentrations measured. One of the standards shall have a concentration equal to or less than the laboratory's lowest reporting concentration for the parameter involved. For colorimetric analyses, a series of five or more non-zero standards for a curve prepared every 12 months or three or more non-zero standards for curves established each day, or standards as set forth in the analytical procedure, shall be analyzed to establish a calibration curve. A manufacturer's factory-set calibration (internal curve) shall be verified with the same number of standards and frequency as a prepared curve.
9	Is a minimum correlation coefficient of 0.995 achieved for calibration curves? [NC WW/GW LC Policy]			When linear regression is used, use the minimum correlation coefficient specified in the method. If the minimum correlation coefficient is not specified, then a minimum value of 0.995 (or a coefficient of determination, r^2, of 0.99) is required.
	PROCEDURE – Sample Preparation	**LAB**	**SOP**	**EXPLANATION**
10	Are samples filtered to remove suspended solids? [SM 4500-NO_2^- B-2011 (1) (b) and (4) (a)]			Remove suspended solids by filtration. If sample contains suspended solids, filter through a 0.45-µm-pore-diam membrane filter. NOTE: If this is required, a filtered blank and laboratory fortified blank must also be analyzed.
11	Is sample pH adjusted to between 5 and 9, when outside this range? [SM 4500- NO_2^- B-2011 (4) (b)]			If sample pH is not between 5 and 9, adjust to that range with 1*N* HCl or NH_4OH as required.
	PROCEDURE – Sample Analysis	**LAB**	**SOP**	**EXPLANATION**
12	What volume of sample is analyzed? [SM 4500- NO_2^- B-2011 (4) (b)] **ANSWER:**			To 50.0 ml sample, or to a portion diluted to 50.0 ml, add 2 ml color regent and mix.
13	Are 2 ml color reagent added to each sample and quality control standard? [SM 4500- NO_2^- B-2011 (4) (b)]			To 50.0 ml sample, or to a portion diluted to 50.0 ml, add 2 ml color regent and mix.
14	Is absorbance of each standard and quality control standard measured at 543 nm between 10 min and 2 hours after adding the color reagent? [SM 4500- NO_2^- B-2011 (4) (c)]			Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm.
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
15	Is each new analyst required to perform an initial demonstration of capability prior to analyzing samples? [SM 4020 B-2011 (1) (a)]			Before new analysts run any samples, verify their capability with the method. Run a laboratory-fortified blank (LFB) (4020B.2e) at least four times and compare to the limits listed in the method.
16	Is a lower reporting limit standard analyzed or back-calculated with each analysis? [15A NCAC 2H .0805 (a) (7) (H)]			Laboratories shall analyze or back-calculate a standard at the same concentration as the lowest reporting concentration each day samples are analyzed.
17	What is the acceptance criterion for the lower reporting limit standard? [15A NCAC 2H .0805 (a) (7) (A)] **ANSWER:**			Unless specified by the method or this Rule, each laboratory shall establish performance acceptance criteria for all quality control analyses.

18	What corrective action does the laboratory take if the lower reporting limit standard does not meet the acceptance criterion? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible. Recalibrate/re-verify the curve
19	When performing an initial calibration, is each calibration point back-calculated and evaluated against established criteria? [SM 4020 B-2011 (2) (a)]			Back calculate the concentration of each calibration point. The back-calculated and true concentration should agree within ±10%, unless different criteria are specified in an individual method. At the lower limit of the operations a range, acceptance criteria are usually wider. Such criteria must be defined in the laboratory's QA plan.
20	Is at least one method blank analyzed with each batch of 20 or fewer samples? [SM 4020 B-2011 (2) (d)]			The reagent/method blank is treated exactly like standards and samples. Include at least one MB daily or with each batch of 20 or fewer samples, whichever is more frequent.
21	Is the method blank concentration less than or equal to ½ the reporting limit? [15A NCAC 2H .0805 (a) (7) (H) (i)]			The concentration of reagent, method, and calibration blanks shall not exceed 50 percent of the lowest reporting concentration or as otherwise specified by the reference method.
22	What corrective action is taken if the method blank is not acceptable? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible.
23	Is a calibration verification standard analyzed initially (i.e., prior to sample analysis), after every tenth sample and at the end of each sample group to check for carry over and calibration drift? [15A NCAC 2H .0805 (a) (7) (H)]			Rule: A calibration blank and calibration verification standard shall be analyzed prior to sample analysis, after every tenth sample, and at the end of each sample group, unless otherwise specified by the method, to check for carryover and calibration drift. **SM states:** Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically, after each batch of ten samples and at the end of the run. The calibration verification standard's analyte concentration should be varied over the calibration range to determine detector response.
24	What is the acceptance criterion for the calibration verification standard? [SM 4020 B-2011 (2) (b)] **ANSWER:**			Results must not exceed ±10% of its true value and calibration blank results must not be > one-half the reporting level (unless the method specifies otherwise).
25	Does the laboratory take appropriate corrective action if the calibration verification standard result exceeds ±10% of the true value? [SM 4020 B-2011 (2) (b)]			**SM states:** If the calibration verification fails, immediately cease analyzing samples and initiate corrective action. Then, re-analyze the calibration verification. If the calibration verification passes continue the analysis. Otherwise, repeat initial calibration and reanalyze samples run since the last acceptable calibration verification.
26	Is a calibration blank analyzed initially (i.e., prior to sample analysis), after every tenth sample and at the end of each sample group to check for carry over and calibration drift? [15A NCAC 2H .0805 (a)			A calibration blank and calibration verification standard shall be analyzed prior to sample analysis, after every tenth sample, and at the

	(7) (H)]			end of each sample group, unless otherwise specified by the method, to check for carryover and calibration drift.
27	What is the acceptance criterion for the calibration blank? [SM 4020 B-2011 (2) (b) and 15A NCAC 2H .0805 (a) (7) (H) (i)] **ANSWER:**			SM: Calibration blank results must not be > one-half the reporting level (unless the method specifies otherwise). Rule: The concentration of reagent, method, and calibration blanks shall not exceed 50 percent of the lowest reporting concentration or as otherwise specified by the reference method.
28	Does the laboratory take appropriate corrective action if the calibration blank results are greater than one-half the reporting level? [SM 4020 B-2011 (2) (b)]			**SM states:** If the calibration verification fails, immediately cease analyzing samples and initiate corrective action. Then, re-analyze the calibration verification. If the calibration verification passes continue the analysis. Otherwise, repeat initial calibration and reanalyze samples run since the last acceptable calibration verification.
29	Does the laboratory analyze a laboratory-fortified blank (LFB) at least daily or per batch of 20 or fewer samples? [SM 4020 B-2011 (2) (e)] **List value(s) and acceptance criterion of standard used.**			The LFB may be either a primary or secondary source standard so it may serve dual roles. If the LFB is **primary source**, it may be equivalent to the CVS (refer to question 23). Analyze at least one daily or per batch of 20 or fewer samples. Use control charts to establish limits or default to the CVS acceptance criterion of ±10%. If the LFB is **secondary source**, it may be equivalent to the second source standard (refer to question 31). Analyze one daily or per batch of 20 or fewer samples. Use control charts to establish limits. **SM states:** LFBs (and LFMs) do not have to be made from a second source (unless the method specifies otherwise) as long as each initial calibration solution is verified via a second source. Ideally, vary LFB concentrations to cover the range from the midpoint to the lower part of calibration curve, including the reporting limit. Include one LFB daily or per each batch of 20 or fewer samples. Calculate %recovery, plot control charts and determine control limits for the LFB unless otherwise specified in the method.
30	What corrective action is taken if the LFB recovery is outside established control limits? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			The concentration of reagent, method, and calibration blanks shall not exceed 50 percent of the lowest reporting concentration or as otherwise specified by the reference method.**SM states**: Establish corrective actions to take if the LFB does not satisfy acceptance criteria.
31	Is a second source standard analyzed after each initial calibration before sample analysis? [15A NCAC 2H .0805 (a) (7) (H) (ii)]			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.
32	Is a Laboratory Fortified Matrix (LFM) analyzed with each batch of 20 or fewer samples? [SM 4020 B-2011 (2) (g)] [SM 4020 B-2011 Table 4020:I]			Laboratory fortified matrix is the same as a matrix spike; that is, a spiked sample. **Note: No option to perform an environmental sample duplicate and then spike separately – must perform MS/MSD for this method.** **SM states:** Include at least one LFM/LFMD daily or with each batch of 20 or fewer samples.

33	Is a Laboratory Fortified Matrix Duplicate (LFMD) analyzed with each batch of 20 or fewer samples? [SM 4020 B-2011 (2) (g)] [SM 4020 B-2011 Table 4020:I]			Laboratory fortified matrix is the same as a matrix spike; that is, a spiked sample. **Note: No option to perform an environmental sample duplicate and then spike separately – must perform MS/MSD for this method.** **SM states:** Include at least one LFM/LFMD daily or with each batch of 20 or fewer samples.
34	How is the LFM prepared? [SM 4020 B-2011 (2) (g)] **ANSWER:**			See Matrix Spike Technical Assistance document. Use the same solution as the LFB for the LFM to evaluate bias attributed to matrix and accuracy of the LFM. **SM states:** To prepare an LFM, add a known concentration of analytes (**ideally** from a second source) to a randomly selected routine sample without increasing its volume by more than 5%. Ideally the new concentration **should be** at or below the midpoint of the calibration curve, and for maximum accuracy, the spike **should** approximately double the sample's original concentration. If necessary, dilute the spiked sample to bring the measurement within the calibration curve. Also rotate the range of spike concentrations to verify performance at various levels. If the spike solution contribution to the fortified sample is kept to 1% or less, a spike dilution correction does not have to be calculated.
35	What is the acceptance criterion for LFM/LFMD recovery? [SM 4020 B-2011 (2) (g)] **ANSWER:**			Will have two % recovery calculations for accuracy from spike recoveries and one RPD calculation for precision from duplicate calculation (see question 41). **SM states:** Calculate recovery limits and RPD, and plot control charts to determine acceptance criteria.
36	What corrective action does the laboratory take if the LFM/LFMD results are outside of established control limits for **accuracy**? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible. Our Rule requires corrective action any time quality control results indicate a problem. Compare to LFB result and other QC. Reanalyze LFM. If it still fails, qualify the spiked sample result. **SM states**: Establish corrective actions to be taken if the LFM does not satisfy acceptance criteria.
37	What is the acceptance criterion for LFM/LFMD for **precision** (i.e., relative percent difference)? [SM 4020 B-2011 (2) (g)] **ANSWER:**			**SM states:** Calculate percent recovery and relative percent difference, plot control charts (unless method specifies acceptance criteria) and determine control limits for spikes at different concentrations. Ensure the method's performance criteria are satisfied. **Bottom line:** We are not requiring control charts but will instead accept a system of trend analysis. That is, the lab's monitoring of the trends in the data. 40 CFR part 136.7 (viii) states: Control charts (or other trend analysis of quality control results).

38	What corrective action does the laboratory take if the LFM/LFMD results are outside of established control limits for **precision**? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible.
39	Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [15A NCAC 2H .0805 (a) (7) (B)]			If the sample cannot be reanalyzed, or if the quality control results continue to fall outside established limits or show an analytical problem, the results shall be qualified as such. If data qualifiers are used to qualify samples not meeting QC requirements, the data may not be useable for the intended purposes. It is the responsibility of the laboratory to provide the client or end-user of the data with sufficient information to determine the usability of the qualified data.

Nitrite-free water: If it is not known that the distilled or demineralized water is free from NO_2^-, use either of the following procedures to prepare nitrite-free water.

1. Add to 1L distilled water one small crystal each of $KMnO_4$ and either $Ba(OH)_2$ or $Ca(OH)_2$. Redistill in an all-borosilicate-glass apparatus and discard the initial 50 ml of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent indicate s the presence of permanganate.
2. Add 1 ml conc. H_2SO_4 and 0.2 ml $MnSO_4$ solution (36.4 g $MnSO_4 \cdot H_2O$/100 ml distilled water) to each 1 L distilled water, and make pink with 1 to 3 ml $KMnO_4$ solution (400 mg $KMnO_4$/L distilled water). Redistill as descried in the preceding paragraph.

Color reagent: To 800 ml water add 100 ml 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

Sodium oxalate, 0.025*M* (0.05*N*): Dissolve 3.350 g $Na_2C_2O_4$, primary standard grade, in water and dilute to 1000 ml.

Ferrous Ammonium Sulfate, 0.05*M* (0.05*N*): Dissolve 19.607 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ plus 20 ml conc H_2SO_4 in water and dilute to 1000 ml. Standardize.

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, discharge permanganate color with a known quantity of standard reductant such as 0.025*M* $Na_2C_2O_4$ 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 t 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 0.05*N* $KMnO_4$, 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 0.025*M* $Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.05*N* $KMnO_4$ 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 0.05*N* $KMnO_4$ 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 NO2- 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 KMnO4 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:

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Inspector: _____Date:_____