NC DENR/DWQ LABORATORY CERTIFICATION

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

Parameter: **Total Phosphorus**
Method: **EPA 365.1, Rev. 2.0 (1993) (Aqueous)**
Automated Colorimetry

EQUIPMENT:

	Automated continuous flow analyzer **Model:** **Wavelength:** 650-660 or 880 nm		Acid-washed glassware
	Filters **Type:** **Pore Size:**		Hotplate
			Water bath, 95°C
	Balance -- Analytical, capable of accurately weighing to the nearest 0.0001 g.		Optional: Autoclave

ANALYSIS REAGENTS: See last page for reagent recipes

	Sulfuric acid (H_2SO_4), 5 *N*		Phenolphthalein indicator solution		Standard phosphorus solution
	Ammonium molybdate solution		Sodium bisulfate ($NaHSO_3$) solution		Stock phosphorus solution
	Antimony potassium tartrate solution		Sulfuric acid (H_2SO_4), 11 *N*		Acid wash water
	Combined reagent		Ammonium persulfate		Ascorbic Acid, 0.1M

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 preserved at time of collection with H_2SO_4 to pH of <2? [40 CFR 136.3 Table II]			Preservation not required if analyzed within 15 minutes.
5	Are samples iced to above freezing but ≤ 6 º C during transport? [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.
6	Is pH checked to document pH <2 upon receipt? [40 CFR 136.3 Table II]			Must be documented, pH paper may be used

7	What action is taken if pH is >2? [15A NCAC 2H .0805 (a) (7) (M)] **ANSWER:**			Sample preservation shall be verified and documented. If a laboratory receives a sample subject to G.S. 143-215.1 and 143-215.63 that does not meet sample collection, holding time, or preservation requirements, the laboratory shall document the incident, notify the sample collector or client, and secure another sample that meets the regulatory requirements, if possible. If another viable sample cannot be secured, the original sample may be analyzed but the results reported shall be qualified with the nature of the sample collection, holding time, or preservation infractions and the laboratory shall notify the State Laboratory of the infractions. The notification shall include a statement indicating corrective action taken to prevent future infractions.
8	Are samples refrigerated above freezing and ≤ 6°C during storage? [40 CFR 136.3 Table II and footnote 18]			
9	Are samples analyzed within 28 days of collection? [40 CFR 136.3 Table II]			
	PROCEDURE – Calibration	**LAB**	**SOP**	**EXPLANATION**
10	What is your laboratory's lower reporting limit? [15A NCAC 2H .0805 (a) (7) (H)] **ANSWER:**			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.
11	Is a calibration curve consisting of at least three standards and a blank analyzed daily or a curve consisting of at least five standards analyzed annually? [15A NCAC 2H .0805 (a) (7) (H) (v)] [EPA Method 365.1, Rev. 2.0 (1993), Section 10.1] **List the calibration standard concentrations:**			Method states: Prepare a series of at least three standards, covering the desired range, and a blank Rules state: 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.
12	Are calibration standards and calibration blanks processed the same as samples? [EPA Method 365.1, Rev. 2.0 (1993), Section 10.2]			Process standards and blanks as described in Section 11.0, Procedure.
13	Are standards analyzed in order of decreasing concentration? [EPA Method 365.1, Rev. 2.0 (1993), Section 10.5]			
14	Does each standard curve have a correlation coefficient ≥0.995? [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 Analysis	**LAB**	**SOP**	**EXPLANATION**
15	What sample volume is analyzed? [EPA Method 365.1, Rev. 2.0 (1993), Section 11.1.1] **ANSWER:**			Transfer 50 mL of sample or an aliquot diluted to 50 mL into a 125 mL Erlenmeyer flask and

16	Is 1 ml of 11*N* sulfuric acid added? [EPA Method 365.1, Rev. 2.0 (1993), Section 11.1.1]			add 1 mL of 11 N sulfuric acid (7.7).
17	Is 0.4 g ammonium persulfate added and the sample mixed? [EPA Method 365.1, Rev. 2.0 (1993), Section 11.1.2]			Add 0.4 g ammonium persulfate (7.8), mix.
18	Is the mixture gently boiled for approximately 30-40 minutes or until a final volume of about 10 mL is reached? [EPA Method 365.1, Rev. 2.0 (1993), Section 11.1.3]			Boil gently for approximately 30-40 minutes or until a final volume of about 10 mL is reached. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi).
19	Is the sample cooled and diluted to approximately 50 ml? [EPA Method 365.1, Rev. 2.0 (1993), Section 11.1.4]			Cool, dilute to approximately 50 mL.
20	If the sample is not clear, is it filtered? [EPA Method 365.1, Rev. 2.0 (1993), Section 11.1.4]			Method does not specify a filter type or pore size.
21	Are samples whose computed value is less than 5% of its immediate predecessor rerun? [EPA Method 365.1, Rev. 2.0 (1993), Section 12.2]			Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
22	Has a Method Detection Limit (MDL) been established? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.2.4]			MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit MDLs should be(recommended) determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.
23	Is a second source standard (QCS) analyzed after each initial calibration prior to sample analysis to verify the calibration? [EPA Method 365.1, Rev. 2.0 (1993), Section 10.7] [EPA Method 365.1, Rev. 2.0 (1993), Section 3.10] [15A NCAC 2H .0805 (a) (7) (H) (ii)]			After the calibration has been established, it must be verified by the analysis of a suitable quality control sample (QCS). The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. 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. All standards are second source when using a factory-set curve, so an additional standard is not needed to meet this requirement.
24	What acceptance criterion is used to evaluate the second-source standard (QCS)? [EPA Method 365.1, Rev. 2.0 (1993), Section 10.7] **ANSWER:**			Must not exceed ±10% of the established QCS value.
25	What corrective action is taken if the second source standard (QCS) recovery is outside of established control limits? [EPA Method 365.1, Rev. 2.0 (1993), Section 10.7] **ANSWER:**			If measurements exceed ±10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.
26	Is a reagent blank analyzed with each batch of samples? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.3.1]			The laboratory must analyze at least one LRB with each batch of samples. The reagent blank must be carried through all steps of analysis, including digestion. If any samples must be filtered as in Question #20, the reagent blank must also be filtered.

27	Is the reagent blank concentration less than or equal to ½ of the lowest calibration standard concentration? [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.
28	What corrective action is taken if the reagent blank is not less than or equal to ½ of the lowest calibration standard concentration? [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.
29	Are the calibration blank and mid-range standard (IPC) analyzed each day, prior to sample analyses, after every tenth sample and at the end of the sample run? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.3.4]			For all determinations, the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run.
30	Is the calibration blank acceptance criterion less than or equal to ½ of the reporting limit? [15A NCAC 2H .0805 (a) (7) (H) (i)]			
31	What corrective action is taken if the calibration blank is not less than or equal to ½ of the reporting limit? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			Reanalyze blank. If still not acceptable, repeat the initial calibration, etc. Once problem is resolved, repeat sample determinations since the last acceptable blank.
32	What is the acceptance criterion for recovery of the mid-range standard (IPC)? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.3.4] **ANSWER:**			Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%.
33	What corrective action is taken if the mid-range standard (IPC) recovery varies by greater than 10%? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.3.4] **ANSWER:**			If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed.
34	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.
35	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.
36	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:**			Recalibrate/re-verify the curve
37	Is a Laboratory Fortified Blank (LFB) analyzed with each set of samples?] [EPA Method 365.1, Rev. 2.0 (1993), Sections 3.4 and 9.3.2]			The laboratory must analyze at least one LFB with each batch of samples. The LFB is an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample (i.e., must be carried through all steps of

				analysis, including digestion). **NOTE: If any samples must be filtered, a filtered LFB must also be analyzed.**
38	What is the acceptance criterion for LFB recovery? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.3.3] **ANSWER:**			The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%.
39	What corrective action does the laboratory take if the LFB results are outside of established control limits? [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.
40	Is a Matrix Spike (MS) analyzed at 10% frequency? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.4.1]			The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples.
41	How is the MS prepared? [NC WW/GW LC Matrix Spike Technical Assistance.] **ANSWER:**			See Matrix Spike Technical Assistance document.
42	What is the acceptance criterion for MS recovery? [EPA Method 365.1, Rev. 2.0 (1993), Section 9.4.2 **ANSWER:**			Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%
43	What corrective action does the laboratory take if the MS results are outside of established control limits? [15A NCAC 2H .0805 (a) (7) (B)] [EPA Method 365.1, Rev. 2.0 (1993), Section 9.4.3] **ANSWER:**			EPA 365.1: "If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related". Rule: 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. 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.
44	At what frequency are duplicates and/or Matrix Spike Duplicates (MSD) analyzed? [15A NCAC 2H .0805 (a) (7) (C)] **ANSWER:**			Except where otherwise specified in an analytical method, laboratories shall analyze five percent of all samples in duplicate to document precision. Laboratories analyzing fewer than 20 samples per month shall analyze one duplicate during each month that samples are analyzed. MSD are recommended when sample concentrations are so low that viable statistics cannot be calculated for precision.
45	What is the acceptance criterion for duplicates and/or MS/MSD? [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.

46	What corrective action does the laboratory take if the duplicate and/or MS/MSD results are outside of established control limits? [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.
47	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.

Analytical Reagents & Standards Prep:

Sulfuric acid 5*N*: Slowly add 70 mL of conc. H_2SO_4 to approximately 400 mL of reagent water. Cool to room temperature and dilute to 500 mL with reagent water.

Sulfuric acid, 11*N*: Slowly add 310 mL of conc. H_2SO_4 to approximately 600 2 4 mL distilled water. Cool and dilute to 1000 mL.

Antimony potassium tartrate solution: Weigh 0.3 g $K(SbO)C_4H_4O_6 \cdot ½\ H_2O$ and dissolve in 50 mL reagent water in 100 mL volumetric flask, dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

Ammonium molybdate solution: Dissolve 4 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 mL reagent water. Store in a plastic bottle at 4°C.

Ascorbic acid, 0.1*M*: Dissolve 1.8 g of ascorbic acid in 100 mL of reagent water. This solution is stable for about a week if prepared with water containing no more than trace amounts of heavy metals and stored at 4°C.

Combined reagent: Mix the above reagents in the following proportions for 100 mL of the mixed reagent: 50 mL of 5N H_2SO_4 (Section 7.2), 5 mL of 2 4 antimony potassium tartrate solution (Section 7.3), 15 mL of ammonium molybdate solution (Section 7.4), and 30 mL of ascorbic acid solution (Section 7.5). Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until the turbidity disappears before processing. This volume is sufficient for a four-hour operation. Since the stability of this solution is limited, it must be freshly prepared for each run. Note: A stable solution can be prepared by not including the ascorbic acid in the combined reagent. If this is done, the mixed reagent (molybdate, tartrate, and acid) is pumped through the distilled water line and the ascorbic acid solution (30 mL of 7.5 diluted to 100 mL with reagent water) through the original mixed reagent line.

Acid wash water: Add 40 mL of sulfuric acid solution (Section 7.7) to 1 L of reagent water and dilute to 2 L. (Not to be used when only orthophosphate is being determined).

Phenolphthalein indicator solution (5 g/L): Dissolve 0.5 g of phenolphthalein (CASRN 77-09-8) in a solution of 50 mL of isopropyl alcohol (CASRN 67-63-0) and 50 mL of reagent water.

Stock phosphorus solution: Dissolve 0.4393 g of predried (105°C for one hour) KH_2PO_4 in distilled water and dilute to 1000 mL.
1.0 mL = 0.1 mg P.

Standard phosphorus solution: Dilute 10 mL of stock phosphorus solution to 100 mL with distilled water. 1.0 mL = 0.01 mg P. Prepare an appropriate series of standards by diluting suitable volumes of standard or stock solutions to 100 mL with distilled water.

Additional Comments:

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Inspector: _____ Date: _____