NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION BRANCH

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

Parameter: **Total Phosphorus**
Automated Ascorbic Acid Reduction Method
Method: **Standard Method 4500-P F – 2021 (Aqueous)**
With Standard Methods 4500 P B (5)-2021 Persulfate Digestion

EQUIPMENT:

	Automated Continuous Flow Analyzer **Manufacturer:** **Model:**		
	Acid-washed glassware		Hot plate
	Autoclave		Micro-Kjeldahl type digestion rack
	Glass scoop		Micro-Kjeldahl flasks

DIGESTION/ANALYSIS REAGENTS: See last page for reagent recipes

	Antimony potassium tartrate solution		Ammonium persulfate
	Ammonium molybdate solution		Phenolphthalein indicator aqueous solution
	Ascorbic acid solution		Stock phosphate solution
	Combined reagent		Intermediate phosphate solution
	Dilute sulfuric acid solution		Standard phosphate solution

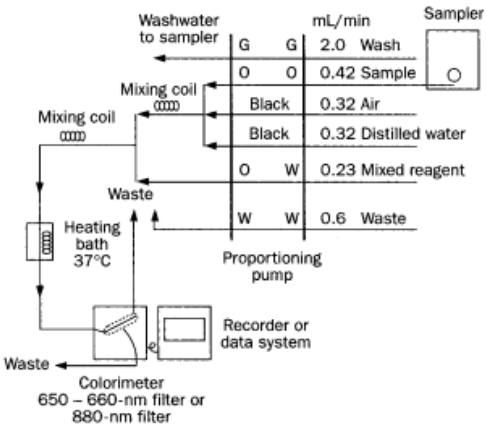
PLEASE COMPLETE CHECKLIST IN INDELIBLE INK

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 revision dates and actions 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 Standard Operating Procedure 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 S.U.? [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 shipment? [40 CFR 136.3 Table II and footnote 18]			
6.	Is pH checked to document pH <2 S.U. upon receipt? [15A NCAC 02H .0805 (a) (7) (M)]			pH paper may be used
7.	What action is taken if pH is >2 S.U.? [15A NCAC 02H .0805 (a) (7) (M)] **Answer:**			If another sample cannot be collected, analyze immediately or adjust pH to <2 S.U. and notify NC WW/GW Certification group that a non-compliant sample was received.

8.	Are samples refrigerated above freezing to ≤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]			
	PERSULFATE DIGESTION	**LAB**	**SOP**	**EXPLANATION**
10.	What volume of sample and standards is digested? [SM 4500-P B-2021 (5) (c)] **Answer:**			The method says this volume is determined by the analytical method, which says to use 50-ml for digestion. Following digestion, it says to dilute the digested sample to a final volume of 100 ml.
11.	Is 0.05 mL (1 drop) of phenolphthalein indicator solution added? [SM 4500-P B-2021 (5) (c)]			
12.	If a red color develops after the addition of phenolphthalein, is H_2SO_4 solution added dropwise to just discharge the color? [SM 4500-P B-2021 (5) (c)] If no color change is observed, skip to question 14.			
13.	After discharging color, is one additional mL of H_2SO_4 solution added? [SM 4500-P B-2021 (5) (c)]			
14.	Is 0.4 g of solid ammonium persulfate added? [SM 4500-P B-2021 (5) (c)]			Either ammonium persulfate or potassium persulfate is added, not both.
15.	Is 0.5 g of solid potassium persulfate added? [SM 4500-P B-2021 (5) (c)]			Either ammonium persulfate or potassium persulfate is be added, not both.
	If an autoclave or pressure cooker is used, skip to question 23			
16.	Is the sample gently boiled on a preheated hotplate? [SM 4500-P B-2021 (5) (c)]			Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds may require as much as 1.5 to 2 h for complete digestion.
17.	How long are samples digested? [SM 4500-P B-2021 (5) (c)] **Answer:**			See above.
18.	To what volume are samples reduced? [SM 4500-P B-2021 (5) (c)] **Answer:**			See above.
19.	Are samples allowed to cool after digestion? [SM 4500-P B-2021 (5) (c)]			
20.	Are cooled samples diluted to 30 mL with reagent water? [SM 4500-P B-2021 (5) (c)]			
21.	Is 0.05 mL (1 drop) of phenolphthalein indicator solution added and neutralized to a faint pink color with NaOH? [SM 4500-P B-2021 (5) (c)]			
22.	To what volume are samples and standards diluted to after digestion? [SM 4500-P B-2021 (5) (c)] **Answer:**			At the end of the digestion, it says to dilute to the digested sample to a final volume of 100 ml.
	Skip to question 28.			
23.	Is the sample heated for 30 minutes in an autoclave or pressure cooker at 98 to 137 kPa? [SM 4500-P B-2021 (5) (c)]			

24.	Are samples allowed to cool after digestion? [SM 4500-P B-2021 (5) (c)]			
25.	Is 0.05 mL (1 drop) of phenolphthalein indicator solution added and neutralized to a faint pink color with 1N NaOH? [SM 4500-P B-2021 (5) (c)]			
26.	What volume are samples and standards diluted to after digestion? [SM 4500-P B-2021 (5) (c)] **Answer:**			At the end of the digestion, it says to dilute to the digested sample to a final volume of 100 ml.
27.	Is information regarding sample ID, initial and final volume, digestion parameters (e.g., pressure, length of time, etc.) documented in a digestion log? [15A NCAC 02H .0805 (a) (7) (E)]			All analytical records, including original observations and information necessary to facilitate historical reconstruction of the calculated results, shall be maintained for five years. All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
	PROCEDURE – Instrument Calibration	**LAB**	**SOP**	**EXPLANATION**
28.	What is the laboratory's lower reporting limit? [15A NCAC 02H .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.
29.	Is an initial calibration performed each time analysis is performed or is a stored calibration used? **Answer:**			
30.	**List the values of standards used for the calibration:** [15A NCAC 02H .0805 (a) (7) (H) (v)] **Answer:**			Rule: 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.
31.	Does each standard curve have a correlation coefficient ≥0.995? [NC WW/GW LCB Correlation Coefficient for Linear Calibration Curves Policy]			
32.	Is the absorbance of each calibration standard compared to the curve and recalculated to determine its concentration? [SM 4020 B-2022 (1) (b)]			
33.	What are the acceptance criteria for the recalculated calibration standards? [SM 4020 B-2022 (1) (b)] **Answer:**			Up to twice the MRL ±50% Between 3 and 5 times the MRL ±20% Greater than 5 times the MRL ±10%
34.	What corrective action is taken if the acceptance criteria are not met for the recalculated calibration standards? [SM 4020 B-2022 (1) (b)] **Answer:**			Identify the source of any outlier(s) and correct before sample quantitation
	PROCEDURE – Sample Analysis	**LAB**	**SOP**	**EXPLANATION**
35.	Is 0.05 ml (1 drop) of phenolphthalein indicator solution added? [SM 4500-P F-2021 (4)]			
36.	If a red color develops after the addition of phenolphthalein, is 5N H_2SO_4 solution added dropwise to just discharge the color? [SM 4500-P F-2021 (4)]			

37.	Is the pH check documented? 15A NCAC 02H .0805 (a) (7) (E)]			All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
38.	Is the manifold setup as shown in SM 4500-P F-2021, Figure 4500-P: 2? [SM 4500-P F-2021 (4)]			 Figure 4500-P:2
39.	Is the digested sample filtered if needed, for turbidity interference? [SM 4500-P F-2021 (1) (b)]			
40.	Are samples analyzed according to the analyzer manufacturer's instructions?			Set up manifold as shown in Figure 4500-P: 2 and follow the general procedure described by the manufacturer.
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
41.	Is all glassware acid-washed as described in SM 4500-P C-2021 (2) (b)? [SM 4500-P F-2021 (2) (c)]			Use acid-washed glassware for determining low concentrations of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.
42.	Has each analyst performing this analysis completed an Initial Demonstration of Proficiency? [15A NCAC 02H .0805 (a) (7) (P) (iii)] **Attach a copy of each analyst's documentation to this checklist.**			Each laboratory shall develop and implement a documented training program that includes documentation that: staff have obtained acceptable results on Proficiency Testing Samples pursuant to Rule .0803(1) of this Section or other demonstrations of proficiency (e.g., side- by-side comparison with a trained analyst, acceptable results on a single-blind performance evaluation sample, an initial demonstration of capability study prescribed by the reference method).
43.	Is the absorbance of each calibration standard compared to the curve and recalculated to determine its concentration? [SM 4020 B-2022 (1) (b)]			
44.	What are the acceptance criteria for the recalculated calibration standards? [SM 4020 B-2022 (1) (b)] **Answer:**			Up to twice the MRL ±50% Between 3 and 5 times the MRL ±20% Greater than 5 times the MRL ±10%
45.	What corrective action is taken if the acceptance criteria are not met for the recalculated calibration standards? [SM 4020 B-2022 (1) (b)] **Answer:**			Identify the source of any outlier(s) and correct before sample quantitation

46.	Is a calibration/reagent blank analyzed prior to sample analysis, after every 10th sample and at the end of the sample group? [15A NCAC 02H .0805 (a) (7) (H)]			The calibration and reagent blank are both digested.
47.	Are all blank concentrations less than or equal to ½ the concentration of the lowest calibration standard? [15A NCAC 02H .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.
48.	What corrective action is taken if any blanks are not acceptable? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			Our Rule requires corrective action any time quality control results indicate a problem.
49.	Is the calibration verified by analyzing a calibration verification standard (CVS) or Continuing Calibration Verification (CCV) prior to sample analysis, after every ten samples and at the end of the run? [SM 4020 B-2022 (1) (c) and 15A NCAC 02H .0805 (a) (7) (H)] **List concentration of standard:**			**Rules state:** 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 analyzing one standard whose concentration is near the midpoint of the calibration range. Other concentrations (e.g., one near the MRL) may be used, but be aware that the acceptance criteria may vary depending on the standard's concentration.
50.	What is the acceptance criterion of the calibration verification standard? [15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			Each laboratory shall establish performance acceptance criteria for all quality control analyses. Each laboratory shall calculate and document the precision and accuracy of all quality control analyses with each sample set.
51.	What corrective action is taken if the calibration verification standard does not meet the acceptance criterion? [15A NCAC 02H .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. 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.
52.	Does the laboratory analyze a second source standard to verify standard preparation? [15A NCAC 02H .0805 (a) (7) (H) (ii)] **List the second source concentration:**			**Rules:** 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. The required Laboratory Fortified Blank (LFB) may serve as the second source standard (refer to question #55)
53.	Is the acceptance criterion for the second source standard ±10% recovery? [SM 4020 B-2022 (1) (b)]			The analytical results for this second source *mid-range standard must be within 10% of its true value. [*the standard is not required to be mid-range]
54.	What corrective action is taken if the second source standard recovery is outside of established control limits? [SM 4020B-2022 (1) (b)] **Answer:**			If not, determine the cause of the error, take corrective action, and re-verify the calibration. If the re-verification passes, continue the analyses; otherwise, repeat the initial calibration.
55.	Does the laboratory analyze a laboratory-fortified blank (LFB) at least daily or per batch of 20 or fewer samples? [SM 4020 B-2022 (6)]			As a minimum, include one LFB with each sample set (batch) or on a 5% basis, whichever is more frequent. Depending on method requirements, prepare the

	List concentration of standard used:			addition solution from either the same reference source used for calibration or an independent source. The LFB is a reagent blank (i.e., treated just like a sample including addition of the preservation acid) fortified with the analyte. If the LFB is **primary source**, it may be equivalent to the CVS/CCV (refer to question #49). 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. If the LFB is **secondary source**, it may be equivalent to the second source standard (refer to question #52). Analyze one daily or per batch of 20 or fewer samples. The acceptance criterion must be recovery within ± 10% of true value.
56.	What is the acceptance criterion of the LFB? [SM 4020 B-2022 (1) (b) and (6)] **Answer:**			If used as the second source verification, the acceptance criterion must be recovery within ± 10% of true value. If primary source, evaluate the LFB for percent recovery of the added analytes by comparing results to method-specified limits, control charts, or other approved criteria.
57.	What corrective action is taken if the LFB recovery is outside established control limits? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			Our Rule requires corrective action any time quality control results indicate a problem.
58.	If a calibration curve is not analyzed each day of analysis, is a lower reporting limit standard analyzed? [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.
59.	What is the acceptance criterion of 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.
60.	What corrective action is taken if the lower reporting limit standard recovery is 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.
61.	Is a Laboratory Fortified Matrix (LFM) analyzed with each batch of 20 or fewer samples? [SM 4020 B-2022 (7) and Table 4020:I]			**SM states:** Include at least one LFM/LFMD daily or with each batch of 20 or fewer samples.
62.	How is the LFM (spike) prepared? [NC WW/GW LCB Matrix Spike Technical Assistance and SM 4020 B-2022 (7)] **Answer:**			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. See Matrix Spike Technical Assistance document.

63.	Is a Laboratory Fortified Matrix Duplicate (LFMD) analyzed with each batch of 20 or fewer samples? [SM 4020 B-2022 (8) and Table 4020:I]			**SM states:** As a minimum, include one duplicate sample or one LFM duplicate with each sample set (batch) or on a 5% basis, whichever is more frequent, and process it independently through the entire sample preparation and analysis Laboratory fortified matrix is the same as a matrix spike; that is, a spiked sample. **Note: Based on Table 4020:I, no option to perform an environmental sample duplicate and then spike separately – must perform MS/MSD for this method.**
64.	What is the acceptance criterion for LFM/LFMD recovery (accuracy)? [15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			Each laboratory shall establish performance acceptance criteria for all quality control analyses. Each laboratory shall calculate and document the precision and accuracy of all quality control analyses with each sample set.
65.	What corrective action does the laboratory take if the LFM/LFMD results are outside of established control limits for **accuracy**? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			Our Rule requires corrective action any time quality control results indicate a problem.
66.	What is the acceptance criterion for LFM/LFMD precision? [15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			Each laboratory shall establish performance acceptance criteria for all quality control analyses. Each laboratory shall calculate and document the precision and accuracy of all quality control analyses with each sample set.
67.	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:**			Our Rule requires corrective action any time quality control results indicate a problem.
68.	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 (e) (5)]			Reported data associated with quality control failures, improper sample collection, holding time exceedances, or improper preservation shall be qualified as such.

Reagents:

Sulfuric acid, H_2SO_4 , 5*N*: Dilute 70 mL conc H_2SO_4 to 500 mL with reagent water.

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

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

Ascorbic acid, 0.1M: Dissolve 1.76 g ascorbic acid in 100 mL reagent water. The solution is stable for about 1 week at 4°C.

Combined reagent: Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5*N* H_2SO_4, 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution. Mix after addition of each reagent. Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. **The reagent is stable for 4 hours.**

Dilute sulfuric acid solution: Slowly add 140 mL conc H_2SO_4 to 600 mL reagent water. When cool, dilute to 1 L.

Ammonium persulfate, $(NH_4)_2S_2O_8$, crystalline.

Phenolphthalein indicator aqueous solution.

Stock phosphate solution: Dissolve 439.3 mg anhydrous KH_2PO_4, dried for 1 h at 105°C, in reagent water and dilute to 1000 mL; 1.00 mL = 100 µg P.

Intermediate phosphate solution: Dilute 100.0 mL stock phosphate solution to 1000 mL with reagent water; 1.00 mL = 10.0 µg P.

Standard phosphate solutions: Prepare a suitable series of standards by diluting appropriate volumes of intermediate phosphate solution.

Additional Comments:

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Inspector: _____Date: _____