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**
Method: **Standard Methods 4500 P E-2021 (Aqueous) Ascorbic Acid Method**
With Standard Methods 4500 P B (5)-2021 Persulfate Digestion

EQUIPMENT:

	Spectrophotometer w/infrared phototube and 2.5 cm light path or longer at 880 nm **Model:**		Filter Photometer w/ red color filter and 0.5 cm light path or longer **Model:**
	Acid-washed glassware		Hot plate
	Autoclave		Micro-Kjeldahl type digestion rack
	Glass scoop		Micro-Kjeldahl flasks

PERSULFATE DIGESTION REAGENTS: **See last page for reagent recipes**

	Phenolphthalein Indicator Solution		Sulfuric Acid Solution		Ammonium Persulfate Solid
	Potassium Persulfate Solid		Sodium Hydroxide, NaOH, 1 *N*		

ANALYSIS REAGENTS: **See last page for reagent recipes**

	Sulfuric acid (H_2SO_4), 5*N*		Ascorbic acid solution		Standard phosphate solution
	Antimony potassium tartrate solution		Combined reagent		
	Ammonium molybdate solution		Stock 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)]			
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 LC Branch that a non-compliant sample was received.
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]			
	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 to 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. Some 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.	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 – Calibration/Calibration Verification	**LAB**	**SOP**	**EXPLANATION**
28.	Is a wavelength of 880 nm used? [SM 4500-P E-2021 (2) (a) (1)]			
29.	What is your 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.
30.	Is the spectrophotometer zeroed with a digested calibration blank prior to calibration? [SM 4500-P E-2021 (4) (c)]			E (4) (a) states: After at least 10 min but no more than 30 min, measure absorbance of each sample at 880-nm, using reagent blank as the reference solution. B (5) says to carry the standards through digestion, which would include the blank 4020 B 5 states: reagent blank (method blank) consists of reagent water (see Section 1080) and all reagents (including preservatives) that typically are in contact with a sample during the entire analytical procedure.

31.	**List the values of the standards used for the calibration.** [SM 4500-P E-2021 (4) (c)] **Standard Concentrations:**			Prepare individual calibration curves from a series of 4 up to 6 standards within the phosphate ranges indicated below. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples. Minimum detectable concentration: Approximately 10 µg P/L. P ranges are as follows: Approximate P Range (mg/L) — Light Path (cm) 0.30 – 2.0 — 0.5 0.15 – 0.30 — 1.0 0.01 – 0.25 — 5.0
32.	Are the calibration standards treated just like samples and carried through the digestion process? [SM 4500-P B-2021 (5) (c)]			Standards must be carried through the same digestion procedure as the samples.
33.	Is the calibration performed for each analysis or is it stored until the calibration verification standard recovery no longer passes, but no longer than 1 year? [SM 4500-P E-2021 (4) (c)] [15A NCAC 02H .0805 (a) (7) (H)] **Answer:**			
34.	If the calibration curve is stored, is the spectrophotometer zeroed with a digested calibration blank prior to sample analysis? [SM 4500-P E-2021 (4) (a)]			
	PROCEDURE – Sample Analysis	**LAB**	**SOP**	**EXPLANATION**
35.	What sample volume is analyzed? [SM 4500-P E-2021 (4) (a)] **Answer:**			
36.	Is 0.05 ml (1 drop) of phenolphthalein indicator solution added? [SM 4500-P E-2021 (4) (a)]			
37.	If a red color develops after the addition of phenolphthalein, is 5*N* H_2SO_4 solution added dropwise to just discharge the color? [SM 4500-P E-2021 (4) (a)]			
38.	Is 8.0 mL of combined reagent added and the sample mixed thoroughly? [SM 4500-P E-2021 (4) (a)]			
39.	Is the absorbance of the sample measured after at least 10 min, but no more than 30 min after adding the combined reagent? [SM 4500-P E-2021 (4) (a)]			
40.	What formula is used to calculate final results? [SM 4500-P E-2021 (5)] **Answer:**			mg P/L = $\frac{\text{mg P (in ~ 58 ml final volume) X 1000}}{\text{ml sample}}$

41.	If necessary, is the sample concentration corrected for turbidity or interfering color with a sample blank? [SM 4500-P E-2021 (4) (b)]			Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a sample blank by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. Subtract blank absorbance from the absorbance of the sample.
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
42.	Is all glassware acid-washed as described in SM 4500-P C-2021 (2) (b)? [SM 4500-P E-2021 (2) (b)]			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.
43.	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).
44.	Is combined reagent made fresh daily & used within 4 hours? [SM 4500-P E-2021 (3) (e)]			Combined reagent is stable for 4 hours.
45.	Does each standard curve have a correlation coefficient ≥0.995? [NC WW/GW LCB Correlation Coefficient for Linear Calibration Curves Policy]			
46.	Is the absorbance of each calibration standard compared to the curve and recalculated to determine its concentration? [SM 4020 B-2022 (1) (b)]			
47.	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%
48.	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
49.	Does the laboratory analyze a second source standard with each initial calibration to verify standard preparation? [SM 4020 B-2022 (1) (b)] [15A NCAC 02H .0805 (a) (7) (H) (ii)] **List the second source standard 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. **SM states:** Verify the initial calibration by analyzing a standard prepared from a different stock standard than that used to

				create the calibration curve; its concentration should be near the midpoint of the calibration range. For a held calibration curve, a second source standard is not required each day samples are analyzed – only with the initial making or verification of the standard curve. The required Laboratory Fortified Blank (LFB) may serve as the second source standard (refer to question #61)
50.	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.
51.	What corrective action is taken if the second source standard recovery is outside of established control limits? [SM 4020 B-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.
52.	Is a calibration 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)]			For this method, the calibration blank and reagent blank are the same
53.	Are all the blank concentrations less than or equal to ½ of the lowest calibration standard concentration? [15A NCAC 02H .0805 (a) (7) (H) (i)]			
54.	What corrective action is taken if any blank is not less than or equal to ½ of the lowest calibration standard concentration? [15A NCAC 02H .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.
55.	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 value(s) of standard used:**			**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.
56.	What is the acceptance criterion of the calibration verification standard? [15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			
57.	What corrective action is taken if the calibration verification standard does not meet the acceptance criterion? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			

58.	Is a standard at the same concentration as the method reporting limit (MRL) analyzed with each analysis? [SM 4020 B-2022 (9)]			With each analytical batch, analyze a reagent-water sample spiked at MRL and ensure that it meets MRL acceptance criteria (generally ±50%).
59.	What is the acceptance criterion of MRL standard? [SM 4020 B-2022 (9)] [15A NCAC 02H .0805 (a) (7) (A)] **ANSWER:**			4020 B states “generally ±50%”. The laboratory must determine the criterion that will be used
60.	What corrective action is taken if the MRL standard doesn’t meet the acceptance criterion? [SM 4020 B-2022 (9)] **Answer:**			If not, re-analyze the entire batch or flag results for all samples in the batch. If the MRL is biased high, non-detect (ND) samples can be reported with flags if the method or regulation allows
61.	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)]			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 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 #55). 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 #49). Analyze one daily or per batch of 20 or fewer samples. The acceptance criterion must be recovery within ± 10% of true value.
62.	Is the LFB primary or secondary source? **Answer:**			See explanation above
63.	What is the concentration and acceptance criterion of the LFB? [15A NCAC 02H .0805 (a) (7) (A) and 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. Otherwise, evaluate the LFB for percent recovery of the added analytes by comparing results to method-specified limits, control charts, or other approved criteria.
64.	What corrective action does the laboratory take if the LFB is outside of the acceptance criterion? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			
65.	Is a Laboratory Fortified Matrix (LFM) analyzed with each sample set, or on a 5% basis, whichever is more frequent? [SM 4020 B-2022			

	(7)]			
66.	How is the LFM prepared? [NC WW/GW LCB Matrix Spike Technical Assistance] [SM 4020 B-2022 (7)] **Answer:**			See Matrix Spike Technical Assistance document. 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.
67.	Is an LFM Duplicate (LFMD) analyzed with each sample set or on a 5% basis, whichever is more frequent? [SM 4020 B-2022 (8) and Table 4020:I]			**SM (8) 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, there is no option to perform an environmental sample duplicate and then spike separately – must perform MS/MSD for this method.**
68.	What is the acceptance criterion for LFM/LFMD recovery (accuracy)? [SM 4020 B-2022 (7) and 15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			There will be two % recovery calculations for accuracy from spike recoveries and one RPD calculation for precision from duplicate calculation. 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.
69.	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) and SM 4020 B-2022 (7)]			Rules state: 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. Compare to LFB result and other QC. Reanalyze LFM. If it still fails, qualify the spiked sample result. 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.
70.	What is the acceptance criterion for LFM/LFMD precision? [SM 4020 B-2022 (8) and 15A NCAC 02H .0805 (a) (7) (A)] **Answer:**			**SM states:** See method for specific acceptance criteria for LFM duplicates or duplicate samples 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. Rule States: 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.
71.	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) and SM 4020 B-2022 (8)] **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. SM states: If duplicate results are out of control, then re-prepare and re-analyze the sample and take additional corrective action, as needed.
72.	Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [15A NCAC 02H (e) (5)]			Reported data associated with quality control failures, improper sample collection, holding time exceedances, or improper preservation shall be qualified as such.

Digestion Reagents:

Sulfuric Acid Solution: Carefully add 300 ml conc H_2SO_4 to 600 mL reagent water and dilute to 1 L with reagent water.

Sodium Hydroxide, 1*N*: Carefully add 40 g of NaOH to 800 ml reagent water. Sir until dissolved and allow to cool. Dilute to 1 L with reagent water.

Sodium Hydroxide, 6*N*: Carefully add 240 g of NaOH to 800 ml reagent water. Sir until dissolved and allow to cool. Dilute to 1 L with reagent water.

Analytical Reagents & Standards Prep:

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 1.3715 g $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$ in 400 mL reagent water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL reagent water. Store in a glass-stoppered bottle.

Ascorbic acid, 0.1*M*: 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, and 30 mL ascorbic acid 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 h.**

Stock phosphate solution*:* Dissolve in reagent water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL; 1.00 mL = 50.0 µg PO_4^{3-}-P.

Standard phosphate solution*:* Dilute 50.0 mL stock phosphate solution to 1000 mL with reagent water; 1.00 mL = 2.50 µg P.

Additional Comments:

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Inspector: _____Date: _____