

# Total Phosphorus - Digestion and Manual Colorimetric Method Checklist \* REV. 11/17/23

Based on NR 149 (2021), NR 219 (2022), and Standard Methods 4500-P B.5, E (1999, 2011)

Some questions may not be applicable to every lab. If applicable, all answers must be "yes" to be in compliance.

**\*This checklist was created for the aid of registered laboratories. It is only an internal audit guideline; it is not meant to be comprehensive of all regulatory requirements, to dictate DNR audit format, or to include all acceptable method options.**

**Laboratories must comply with all applicable code and method requirements whether listed on this checklist or not. Additional general NR 149 requirements are on a separate checklist.**

	Sample Storage and Handling	Y	N	Notes	Reference
1	If preparation/analysis is not started immediately ( $\leq 15$ minutes), are the samples stored at $\leq 6^{\circ}\text{C}$ (but above its freezing point) prior to analysis?				NR 219 Table F; NR 149.442 (4)(b)
2	If preparation/analysis is not started immediately ( $\leq 15$ minutes), are the samples preserved using sulfuric acid?				NR 219 Table F (footnote 2)
3	When samples are preserved, is the pH measured to ensure it is $< 2$ ?				NR 149.442 (2)(e)
4	Are preserved samples analyzed within the hold time of 28 days?				NR 219 Table F

	Reagents and Standards	Y	N	Notes	Reference
5	Is the ascorbic acid only stored for up to a week and stored refrigerated?				SM 4500-P E (3)(d)
6	Is the combined reagent made fresh each day of use and used within 4 hours?				SM 4500-P E (3)(e)
7	If any reagent (e.g., antimony potassium tartrate solution, ammonium molybdate solution, combined color reagent) is prepared in the lab, is it made according to the instructions in the method?				SM 4500-P E (3)
8	Are reagents and standards unexpired?				NR 149.39 (3)(d)
9	Are reagents properly labeled (with chemical name, concentration, and expiration date)?				NR 149.39 (3)(a)

	Equipment	Y	N	Notes	Reference
10	Is glassware cleaned sufficiently enough that all method blanks pass?				SM 4500-P C (2)(b), E (2)(b)
11	Is phosphate free soap and distilled or deionized water used to clean glassware? ( <i>some tap water may contain polyphosphates that affects phosphorus results</i> )				SM 4500-P C (2)(b), E (2)(b)
12	Is a hot plate, hot block, or autoclave used to digest samples?				SM 4500-P B (5)(a)
13	If an autoclave is used, is it able to maintain a pressure of 98 - 137 kPa (15 - 19 psi) during digestion? ( <i>this may be demonstrated with the use of a min/max thermometer</i> )				SM 4500-P B (5)(a)(2), (5)(c); NR 149.44 (3)(a)
14	Is absorbance measured at the appropriate wavelength (880 nm)?				SM 4500-P E (2)(a)

	Digestion and Sample Measurement	Y	N	Notes	Reference
15	Is the sample volume accurately measured with a graduated cylinder, volumetric flask, or class A pipette?				NR 149.44 (1)

16	After the sample is mixed and measured into a flask, is phenolphthalein indicator added? If a red color appears, is sulfuric acid solution added until it clears?				SM 4500-P B (5)(c)
17	Are sulfuric acid and persulfate reagents added to the sample in volumes that are proportional to those required by the method? (e.g., 1 mL of 11N H <sub>2</sub> SO <sub>4</sub> and ~0.4 g ammonium persulfate to 50 mL of sample)				SM 4500-P B (5)(c)
18	If a hot plate or hot block is used, are samples gently boiled on a hot plate for 30-40 minutes or until the volume is 1/5 of the starting volume?				SM 4500-P B (5)(c)
19	If a hot plate or hot block is used, if a sample goes dry during digestion, is a new portion of sample used and redigested?				SM 4500-P B (5)(c)
20	If an autoclave is used, are samples heated for 30 minutes at 98 to 137 kPa?				SM 4500-P B (5)(c)
21	Are the sample then cooled, phenolphthalein added, and then neutralized with 1N NaOH to a faint pink color?				SM 4500-P B (5)(c)
22	Is the final volume accurately measured? ( <i>i.e., don't use beakers or Erlenmeyer flasks to measure final volume</i> )				NR 149.44 (1)
23	Is 5N sulfuric acid added dropwise just until the solution is clear?				SM 4500-P E (4)(a)
24	Is the combined color reagent added to the sample in a volume proportional to that required by the method? (e.g., 8 mL of combined reagent to 50 mL of sample)				SM 4500-P E (4)(a)
25	Is an instrument blank (without color reagent) used to zero the spectrophotometer before standards or samples are measured?				NR 149.444 (1)(e)
26	Is the sample absorbance measured between 10 and 30 minutes after the addition of the combined reagent?				SM 4500-P E (4)(a)
27	If the sample response is above the response of the highest calibration standard, is the sample diluted and re-analyzed?				NR 149.47 (1)(c)
28	When samples require dilution, are they diluted before the color reagents are added and then redigested? ( <i>If only a portion of the digested samples had color reagent added, the other portion may be diluted, reacted with color reagent, and analyzed.</i> )				NR 149.50 (2)(d)
29	If a sample is highly colored or turbid, is the absorbance of the sample without color reagent measured and recorded and then the concentration subtracted from the reacted sample concentration?				NR 149.45; SM 4500-P E (4)(b)
30	The method blank is NOT used to adjust the sample results, correct?				NR 149.48 (1)(b)

	Calibration	Y	N	Notes	Reference
31	Is the calibration curve redone if there are two consecutive CCV failures, the instrument leaves the lab, after non-routine maintenance, or conditions change the expected behavior of the instrument?				NR 149.44 (4)(d), NR 149.444 (1)(c), NR 149.446 (5)
32	Are at least 3 non-zero standards used for a linear calibration curve or at least 5 non-zero standards used for a quadratic curve?				NR 149.444 (2), (2)(e)

33	Does the curve include a calibration blank that is treated the same as the other calibration standards and uses the instrument response?				NR 149.50 (2)(a)
34	Is the calibration curve not forced through zero?				NR 149.444 (4)(d)
35	Does the curve have a correlation coefficient (r) of at least 0.995 for a linear curve or a coefficient of determination (r <sup>2</sup> ) of at least 0.995 for a quadratic curve?				NR 149.444 (6)(e), (6)(f)

	Calibration Verification and Quality Control	Y	N	Notes	Reference
36	Is the phosphorus glassware rotated so that the QC are not always in the same container?				NR 149.48 (1)(a)
37	Is a second source standard (ICV) always analyzed after the initial calibration and before samples are analyzed?				NR 149.444 (7)
38	Is the ICV within 90 - 110% of the true value unless otherwise specified in the method?				NR 149.444 (7)(a)
39	Is the CCV performed first (before method blanks and samples) on non-calibration days?				NR 149.446 (1), (2)(a)
40	If more than 20 samples are analyzed in a batch, is a CCV analyzed after the 20th sample?				NR 149.446 (2)(b)
41	Is the CCV within 90 - 110% of the true value?				NR 149.446 (4)(a)
42	If the initial calibration standards are digested, are the ICV and CCV also digested?				NR 149.444 (7), NR 149.446 (1)(b)
43	Is a method blank processed with each sample batch (up to 20 samples) in the same manner as the samples?				NR 149.48 (5)(a), (5)(b)
44	Is the method blank less than the highest of the LOD, 5% of the regulatory limit, or 10% of the sample concentrations?				NR 149.48 (5)(d)

	Reporting and Qualifiers	Y	N	Notes	Reference
45	Are samples results that are less than the MDL reported on the eDMR as "<" the MDL value?				NR 149.47 (1)(a); WPDES Permit
46	Are all reported MDLs adjusted for any dilution (i.e., adjust when the sample amounts used are different than those used for the MDL determination)?				NR 149.48 (2)(d)
47	Are results qualified if samples were analyzed past hold time? ( <i>If preserved, hold time is 28 days; if unpreserved, digestion/analysis must be started within 15 minutes of collection</i> )				NR 219 Table F; NR 149.47 (4)(b)
48	If using an autoclave, are results qualified if samples were not digested for 30 minutes at 98 to 137 kPa?				NR 149.47 (5); SM 4500-P B (5)(c)
49	Are results qualified if the method blank fails?				NR 149.47 (5), NR 149.48 (5)(d)

	Documentation and Records - Are all of the following documented or recorded, if applicable?	Y	N	Notes	Reference
50	Sample collection date				NR 149.45
51	Acid preservation verification				NR 149.45
52	Sample storage temperature				NR 149.45
53	Digestion date				NR 149.45

54	Digestion analyst				NR 149.45
55	Digestion pressure (if an autoclave is used)				NR 149.45
56	Analyst				NR 149.45
57	Analysis date				NR 149.45
58	Unique sample IDs				NR 149.442 (1)(d); NR 149.45
59	Lot or lab ID of standards				NR 149.45
60	Lot or lab ID of reagents				NR 149.45
61	Sample neutralization				NR 149.45
62	Sample volume				NR 149.45
63	No prefilled volumes				NR 149.45
64	Raw data (absorbance)				NR 149.45
65	Units (e.g., mL, abs)				NR 149.45
66	Sequence of analysis is clear				NR 149.45
67	Calibration identification (e.g., date or link to calibration data)				NR 149.45
68	Corrections made to data were done properly (crossed out with a single line; not scribbled out or overwritten)				NR 149.39 (1)(g), NR 149.45
69	Corrective actions taken (e.g., when temperatures or pressures are out of range, analyzed past hold time, QC failures, etc.)				NR 149.38 (3), NR 149.45
70	Instrument maintenance				NR 149.45

Other Observations

WI DNR Total Phosphorus Resources
DNR Website (which includes the resources below): <a href="#">Laboratory Certification   Wisconsin DNR</a>
Example Phosphorus by SM 4500-P B.5, E SOP template
Example total phosphorus colorimetric benchsheets (includes standard preparation calculator)
Example thermometer annual verification log
Example digestion block annual temperature verification log
Example total phosphorus and ammonia preservation and neutralization tracking log
Example daily equipment temperature measurements log
Example auto-pipette quarterly verification log
Example equipment maintenance log
Example prepared and purchased chemical tracking logs
Example general corrective action log
Lab Accreditation Program staff - contact any staff with questions or concerns, especially if there are ongoing QC issues