NC DENR/DWQ LABORATORY CERTIFICATION

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

Parameter: **BIOCHEMICAL OXYGEN DEMAND ($BOD_5/CBOD_5$)**
Method: **5–Day BOD Test – DO Probe**

METHOD OF ANALYSIS:

	Standard Methods 18th Edition: 5210 B ☐
	Standard Methods 19th Edition: 5210 B ☐
	Standard Methods 20th Edition: 5210 B ☐
	Standard Methods Online 5210 B-01 (SM 21st) ☐

EQUIPMENT:

	Air incubator or water bath		DO meter (Model):
	Incubation bottles, 60 mL or 300 mL		DO probe **(circle one)**: membrane probe or LDO probe (Luminescence)
	pH meter (Model):		

	PLEASE COMPLETE CHECKLIST IN INDELIBLE INK	Y	N	EXPLANATION
	PRESERVATION and STORAGE			
1	Are samples iced to above freezing but ≤ 6 ºC during shipment? [40 CFR 136 Table II]			
2	Are samples refrigerated to above freezing but ≤ 6 ºC during storage? [40 CFR 136 Table II]			
3	Are samples analyzed within 48 hrs of collection? [40 CFR 136 Table II]			Time starts from time of last aliquot of composite sample. If Mon., Tues., and Wed. samples are set up on Wed., compare the sample collection time for Mon. and the time in incubator. Sample must be in the incubator by that same time on Wed.
	PROCEDURE – Meter Calibration			
4	How is the DO meter calibrated? [SM 18th, 19th 5210 B. (4) (g). SM 20th 5210 B. (4) (f) (2). SM 21st B. (5) (g).]			References DO method 4500-O G. (Membrane Probe). Calibrate DO/LDO meter per manufacturer's instructions. Generally DO will be air calibration based upon temperature, and elevation or barometric pressure. **SM 20th** BOD method recommends calibration by Azide Modification method – (Winkler Titration Method 4500-O C.) **SM 21st** drops Winkler language. LDO probe must have calibration verified each day of use. Can be performed by back calculating the theoretical DO for the current air calibration conditions (temperature, elevation, barometric pressure, etc). **See attached LDO Daily Check handout.**
5	Is the meter calibration documented on day one and day five? [15A NCAC 2H .0805 (a) (7).]			
6	Is the DO meter calibration checked for drift during and at the end of the sample set? [NC WW/GW LC policy based upon SM 21st 5210 B. (5) (g).]			**SM 18th, 19th, and 20th** References SM 4500-O G. (1) (a). **SM 21st** specifically calls for drift check. SM 18th, 19th, 20th 5210 B. (4) (g). SM 21st 5210 B. (5) (g). Drift checks through out the sample run are recommended for large sample sets.
7	Is the meter calibration drift check documented? [15A NCAC 2H .0805 (a) (7).]			
8	Do the drift checks agree within ± 0.2 mg/L? [NC WW/GW LC policy]			

9	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (F).]			Meter must be recalibrated. Any bottles read since the last acceptable drift check must be reread.
	PROCEDURE – Dilution Water			
10	What is the source of dilution water? [SM 18th, 19th, 20th 5210 B. (4) (a). SM 21st 5210 B. (4) (c).]			Use demineralized, distilled, tap, or natural water. Basically any water source is OK as long as QC is met.
11	Is dilution water aged / conditioned? [SM 20th 5210 B. (4) (b). SM 21st 5210 B. (4) (c).]			Ageing water may improve blanks. Do whatever works for that lab. Do not add nutrients to water until ready to use. (Method states no more than 24 hrs prior to use.) Store water at least overnight in an incubator.
12	If so, for how long? [SM 20th 5210 B. (4) (b). SM 21st 5210 B. (4) (c).]			
13	Is the temperature of the dilution water approximately 20 °C before usage? [SM 18th, 19th, 20th 5210 B. (4) (a). SM 21st 5210 B. (5) (a).]			**SM 18th and 19th** state 20 °C, **SM 20th, 21st** state 20 ± 3 °C. Do not worry too much about what exact temp is - look at initial DOs of blanks – must be 7-9 mg/L **(see question #41)**
14	Are the nutrient solutions prepared or purchased? [SM 5210 B. (3).]			Phosphate buffer, Magnesium sulfate, Calcium chloride, Ferric chloride
15	If purchased – pillows or individual bottles? [SM 5210 B. (3).]			
16	Is the phosphate buffer, prepared or purchased, documented to be pH 7.2? [SM 5210 B. (3) (a).]			Purchased will be documented to be 7.2 on bottle or with pillows. If made: one recipe in **SM 18th and 19th** must check and be 7.2 without adjustment. An additional recipe is included **in SM 20th and 21st** - can be adjusted if not 7.2 pH. **SM 18th and 19th:** 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl to 1 L. **SM 20th and 21st:** 42.5 g KH_2PO_4 and 1.7 g NH_4Cl **or** 54.3 g K_2HPO_4 to 1 L. (This can be adjusted to pH 7.2 with 30% NaOH – dissolve in ≈ 700 mL prior to dilution to 1 L). **NOTE:** Based on the acknowledgements in later editions of SM, it is acceptable to make pH adjustments when citing any edition of SM but preparing as in 20th and 21st editions.
17	Are nutrients free of precipitation or biological growth? [SM 5210 B. (3).]			Liquid reagents are usually stored in a refrigerator (recommended) but are not required to be. If using liquid reagents, there will be 4 bottles.
18	Are nutrients added at rate of 1 mL each per liter of dilution water? [SM 18th, 19th, 20th 5210 B. (4) (a). SM 21st 5210 B. (5) (a).]			Pillows are formulated for different volumes of water – check to be sure the proper pillow is used for the volume of dilution water being made. Pillow contains all 4 reagents.
	PROCEDURE – Sample Preparation			
19	Are samples adjusted to 20 ± 3 °C before analysis? [NC WW/GW LC policy based upon SM 21st 5210 B. (5) (b).]			**SM 18th, 19th, 20th** state ± 1 °C [SM 18th, 19th, 20th 5210 B. (4) (e) (5).] **SM 21st** states ± 3 °C [SM 21st 5210 B. (5) (b).] **NC WW/GW LC policy per SM 21st 5210 B. (5) (b). is 20 ± 3 °C** It is not critical what the exact temperature is. Generally just warm up. As long as initial DO is OK. **(See question #41.)**
20	Is each sample pH checked before analysis and adjusted if needed? [NC WW/GW LC policy based upon SM 20th 5210 B. (4) (e) (1).]			**NC WW/GW LC policy is based upon SM 20th 5210 B. (4) (e) (1). because it is the most liberal range allowed (6.0-8.5) and the most liberal adjusted range allowed (6.5-7.5).** **SM 18th and 19th**: states "if sample has high alkalinity or acidity" adjust to 6.5-7.5

				but never defined what high alkalinity or acidity was. **SM 20th defines high alkalinity as 8.5 pH and acidity as 6.0. If samples are outside that range adjust to 6.5-7.5** SM 18th, 19th, 20th 5210 B. (4) (e) (1). **SM 21st**: if samples are not between 6.0 and 8.0 adjust to 7.0-7.2. 21st 5210 B. (4) (b) (1).
21	What pH criterion is used? **LIST range for adjustment**: [NC WW/GW LC policy based upon SM 20th 5210 B. (4) (e) (1).]			See # 20
22	Is each pH adjustment documented? [15A NCAC 2H .0805 (a) (7).]			The sample pH must be document. For those that require adjustment the initial pH and the final pH must be documented.
23	Are all samples checked for the presence of residual chlorine? [SM 18th, 19th, 20th 5210 B. (4) (e) (2). SM 21st 5210 B. (4) (b) (2).]			O-R (Oxidizing/Reducing agents) testing is not in the methods. Adding **Potassium Bi-iodate** (not listed in method with chemicals used in test) to neutralize reducing agents is old practice. **Is not in method – should not be done**. This is especially true with wide spread use of chlorination/dechlorination we have today. Any excess dechlorination chemicals from the treatment process can add oxygen demand. Since those chemicals are a part of the sample being discharged, their BOD impact on the receiving stream should be included in the result.
24	How are the samples checked for chlorine? [SM 18th, 19th, 20th 5210 B. (4) (e) (2). SM 21st 5210 B. (4) (b) (2).]			It is OK to prescreen samples visually with DPD powder. If no pink color is present, document as no chlorine present. If pink color is present, sample aliquot must be titrated per method. TRC test strips may be used for samples where interference with DPD precludes their use.
25	If chlorine is present, is it properly neutralized with sodium sulfite? [SM 18th, 19th, 20th 5210 B. (4) (e) (2). SM 21st 5210 B. (4) (b) (2).]			**See Neutralizing TRC in BOD Samples document.**
26	Is this documented? [15A NCAC 2H .0805 (a) (7).]			The amount (usually expressed as drops) of sodium sulfite must be documented.
27	How often is sodium sulfite prepared? [SM 5210 B. (3) (f).]			Method says prepare daily but once per week is OK. Just don't want sulfite so weak that such a large volume is added that it becomes a dilution issue. For super chlorinated samples you may need to make a stronger sulfite solution to avoid having to add so much it becomes a dilution issue.
28	Is hydrogen peroxide (H_2O_2) present in any samples? [SM 21st 5210 B. (4) (b) (5).]			Not addressed in 18th, 19th, 20th SM 5210 B.
29	If so, how is hydrogen peroxide treated? [NC WW/GW LC policy based upon SM 21st 5210 B. (4) (b) (5).)			**SM 21st** - H_2O_2 can cause supersaturated DOs. May see higher DO after 5 days than initial DO. **Treatment** – Mix samples vigorously in open container until H_2O_2 dissipates. Check adequacy of H_2O_2 removal by observing DO concentrations over time during mixing or use peroxide specific test strips. Mixing may take 1-2 hrs. The peroxide reaction can be considered complete when the DO no longer increases during a 30-minute period without mixing. Seen predominantly in pretreatment samples.
	PROCEDURE - Seeding			
30	Are samples seeded if required? [SM 18th, 19th, 20th 5210 B. (4) (d) (1). SM 21st 5210 B. (5) (d).]			Samples needing seeding: with **pHs** outside acceptable range, **disinfected** (UV, chlorine, etc.), **industrial**. All **$CBOD_5$** samples must be seeded: See Question # 40 [SM 21st 5210 B. (5) (e).] May just

				seed all samples - that's OK.
31	What is the source of the seeding material? [SM 18th, 19th, 20th, 5210 B. (4) (d) (1). SM 21st 5210 B. (4) (d).]			Can use commercial seed, influent from domestic source, receiving waters. Expanded language in SM 21st Edition, including use of commercial seed.
32	If commercial seed (e.g., Polyseed®, BioSeed®, etc.) is used, how is it prepared? [Manufacturer's instructions and SM 21st 5210 B. (4) (d).]			**See Preparation of Synthetic Seed Material document.**
33	List volumes (mL) of seed for each seed control sample.			Must set a minimum of 2 seed controls (different dilutions), preferably 3. If setting 2 and they are both consistently acceptable (i.e., meet 2 and 1 rule) that is OK.
34	Is sufficient seed material used in each seed control to yield a seed correction of 0.6 - 1.0 mg/L? [SM 18th, 19th, 20th 5210 B. (4) (d) (2). SM 21st 5210 B. (5) (d).]			**SM 20th and 21st** allows for Seed Correction Factor (SCF) greater than 1.0 mg/L but ONLY if lab has data on file to show this is the only way to get acceptable results on GGA – NC WW/GW LC Policy. See section 6 of method. SCF in this case should not exceed 1.4 mg/L (per EPA Region IV) - NC WW/GW LC Policy. You do not want the portion of the DO usage from the seeding material to be too much greater than the DO depletion from the sample. That is, if a sample needs a minimum of 2.0 mg/L to be a "good" depletion than with a SCF of 0.9 mg/L, the portion of the total depletion (2.0 mg/L) that came from the sample would be 1.1 mg/L (2.0 - 0.9 = 1.1 mg/L).
35	Is the seed correction calculated correctly from the seed controls? [SM 18th, 19th, 20th 5210 B. (5). SM 21st 5210 B. (70 (a) (1).]			**See Calculations and Reporting document.**
36	Is the criterion of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L used for the seed correction calculation? [SM 18th, 19th, 20th 5210 B. (5). SM 21st 5210 B. (7) (a) (1).]			
37	Are the results of all acceptable seed control analyses averaged? [SM 18th, 19th, 20th 5210 B. (5). SM 21st 5210 B. (7) (a) (1).]			Calculate each dilution's correction factor individually. Average the results from all "good dilutions" (meet 2 and 1 rule) for the FINAL seed correction factor. If only one dilution is good on routine basis, dilutions need to be adjusted. SM 21st states that if seed control dilutions show a wide variation (± 30%) in depletions per mL of seed, corrective action must be taken. May indicate the presence of toxic substances or large particulates in the seed material.
	PROCEDURE- Sample Analysis			
38	If $CBOD_5$ is required, is a nitrification inhibitor added to all samples, seed controls and GGAs? [SM 18th, 19th, 20th 5210 B. (4) (e) (6). SM 21st 5210 B. (5) (e). and 5210 B. (8).]			We often get questions on acceptance criterion for GGA for $CBOD_5$. Same as without inhibitor:198 ± 30.5 mg/L. **NOTE:** Nitrification inhibitor must NOT be added to blank per SM 21st 5210 B. (6) (c).
39	What nitrification inhibitor is used for $CBOD_5$ samples? [SM 18th, 19th, 20th 5210 B. (4) (e) (6). SM 21st 5210 B. (5) (e).]			TCMP is preferred nitrification inhibitor. SM 21st allows use of ATU (allylthiourea solution) in addition to TCMP (2-chloro-6-trichloromethyl pyridine) as inhibitor. ATU solution is stable for no more than two weeks. Concentrations above 2 mg/L may cause increases in carbonaceous BOD measurements (false high answer).
40	Are all samples, seed controls and GGAs for $CBOD_5$ seeded? [NC WW/GW LC policy based upon SM 21st 5210 B. (5) (e).]			
41	Is initial DO of all bottles between 7 and 9 mg/L? [SM 18th, 19th, 20th, 5210 B. (6) (b). SM 21st 5210 B. (8) (b).]			Will be determined by combination of initial DOs of sample and dilution water. Check blank for initial DO of dilution water. Look at large sample volume

				bottles (300 mLs sample) in cold weather to see if 9.0 mg/L requirement is being met. At 20 °C (incubator temperature) DO saturation is 9.2 mg/L. Any initial DO > 9.0 mg/L may be lost during incubation and yield a false high value. SM 21st 5210 B. 5. a. states the initial DO of the blank must be at least 7.5 mg/L. **NC WW/GW LC policy is the blank must be 7-9 mg/L**
42	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (F).]			Need to warm up samples and agitate vigorously. Usually not enough agitation.
43	Are initial DOs measured within 30 minutes of preparing dilutions? [NC WW/GW LC policy based upon SM 20th 5210 B. (4) (g). and SM 21st 5210 B. (5) (g).]			**SM 18th, 19th**, state time not critical if no Immediate Oxygen Demand (IOD). **SM 20th** states should be within 30 min if no IOD. **SM 21st** states must be within 30 minutes and drops the IOD language. IOD is characterized by abnormally low initial DO. Appears language removed based upon requirement of initial DO must be > 7 – if that is met then considered OK – if not because of IOD would need to re-aerate to above 7 initial DO and read and stopper bottle immediately.
44	Is the probe rinsed between bottles to prevent contamination? [NC WW/GW LC policy based upon SM 18th, 19th, 20th 5210 B. (4) (f) (2).]			Not mentioned in **SM 21st**. May also read each sample's bottles from weaker to stronger without rinsing between each bottle.
45	How many dilutions are set for samples? [SM 18th, 19th, 20th 5210 B. (4) (f). SM 21st 5210 B. (5) (c).]			**SM 18th, 19th**: say "dilutions" (which we interpret as a minimum of two) **SM 20th** states set sufficient dilutions to get two good dilutions (meet 2 and 1 rule). **SM 21st** - minimum 3 dilutions. **NC WW/GW LC policy is a minimum of 2 dilutions must be analyzed. NOTE:** When setting only 2 dilutions, both should be acceptable most of the time. If not, either adjust dilutions or set additional dilution(s). Does not apply when setting 100% sample.
46	For dilutions with >67% sample (> 201 mL) are extra nutrients added? [NC WW/GW LC policy based upon SM 20th 5210 B. (4) (f). and SM 21st 5210 B. (5) (c) (2).]			Best way to do this is use pillows made for 300 mL bottle size. Add 1 pillow to each bottle containing more than 201 mL sample.
47	Are the samples incubated for five days ± 6 hours? [NC WW/GW LC policy based upon SM 21st 5210 B. (5) (h) and (i).]			**SM 21st** defined as ± 6 hours.
48	Are samples incubated in the dark at 20 ± 1 °C? [SM 18th. 19th, 20th 5210 B. (4) (i). SM 21st 5210 B. (5) (h).]			
49	Are water seals maintained on the bottles during incubation? [SM 18th, 19th, 20th, 5210 B. (4) (i). SM 21st 5210 B. (5) (f).]			Make sure caps are used on bottles. If water seals are not maintained air bubbles may form in bottles.
50	Are sufficient sample dilutions analyzed to meet a residual DO of 1.0 mg/L and a DO depletion of at least 2.0 mg/L? [SM 18th, 19th, 20th, 5210 B. (4) (f). SM 21st 5210 B. (5) (c).]			
51	Are all dilutions meeting this criterion averaged for final BOD results? [SM 18th, 19th, 20th, 5210 B. (5). SM 21st 5210 B. (7) (b).]			
52	Are seed corrections properly subtracted from the seeded samples? [SM 18th, 19th, 20th, 5210 B. (5). SM 21st 5210 B. (7) (a) (1).]			**See Calculations and Reporting document.**
53	Is the final BOD of the samples properly calculated? [SM 18th, 19th, 20th, 5210 B. (5). SM 21st 5210 B. (7) (a) (1).]			**See Calculations and Reporting document.**
	QUALITY CONTROL			
54	Is the date/time samples are setup documented? [15A NCAC 2H .0805 (a) (7) (H).]			
55	Is the date/time samples are taken out of the incubator documented? [15A NCAC 2H .0805 (a) (7) (H).]			
56	Are incubator temperatures documented? [15A NCAC 2H .0805 (a) (7) (J).]			20 ± 1 °C
56	Is a dilution water blank analyzed? [SM 18th, 19th, 20th, 5210 B. (4)			

	(h). SM 21st 5210 B. (6) (c).]			
57	Is the dilution water blank depletion ≤ 0.20 mg/L? [NC WW/GW LC policy based upon SM 21st 5210 B. (6) (c).]			**SM 18th, 19th, 20th** state ≤ 0.2 mg/L, preferably ≤ 0.1 mg/L. [SM 18th, 19th, 20th 5210 B. (4) (h). **SM 21st** states ≤ 0.20 mg/L preferably ≤ 0.10 mg/L. SM 21st 5210 B. (6) (c). **NC WW/GW LC policy is ≤ 0.20 mg/L**. This means readings should be made to two decimal places. (An exception may be with analog meters which are not this sensitive - it is recommended that these be replaced when budgets allow. Sample results associated with blanks reading >0.20 mg/L must be qualified. **See Flagging QC Failures document.**)
58	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (F).]			
59	Is a glucose-glutamic acid standard (GGA) analyzed with each set of samples? [SM 18th, 19th, 20th 5210 B. (4) (c). SM 21st 5210 B. (6) (b).]			
60	What is the source of the GGA standard? [SM 18th, 19th, 20th 5210 B. (3) (h). and (4) (c). SM 21st 5210 B. (3) (h). and (6) (b).]			May make or buy prepared. If making, dry reagents at 103 °C for 1 hr.
61	How frequently is the GGA standard made? [NC WW/GW LC policy based upon SM 21st 5210 B. (3) (h).]			**May be held: NC WW/GW LC policy based upon SM 21st 5210 B. (3) (h).** **SM 18th, 19th, 20th** state make fresh daily. **SM 21st** states may be held if maintained in sterile conditions and stored at ≤ 4 °C (≤ 6 °C is okay). Recommendations: Sterilize the water/bottle before making and never place pipet in storage bottle. Pour an aliquot into a small beaker, let warm to room temperature, pipet 6 mLs into sample bottle then discard any excess. Should not try to hold longer than approximately 1 month.
62	Is the GGA standard analyzed at a 2% dilution? [SM 18th, 19th, 20th 5210 B. (4) (c). SM 21st 5210 B. (6) (b).]			Method says must be 6 mL into 300 mL bottle, strength must be 150 mg glucose/L and 150 mg glutamic acid/L. If commercial solution is prepared to a different concentration, adjust dosage used accordingly, for example Hach product is twice as strong. OK to use if adjust dosage by pipetting 3 mLs into bottle, not OK to pipette 6 mls into bottle and divide answer obtained by two.
63	Is the GGA standard seeded? [SM 18th, 19th, 20th 5210 B. (6). SM 21st 5210 B. (8).]			Never have only GGA and seed in bottle-bacteria in seed will begin to consume sugars (glucose) immediately. Always have some dilution water in bottle before adding GGA and seed.
64	Is the value of the GGA standard 198 ± 30.5 mg/L? [NC WW/GW LC policy based upon SM 20th 5210 B. (6).			**SM 21st** states you analyze three GGAs and the average of the three - must be 198 ± 30.5 mg/L. SM 21st 5210 B. (8). **NC WW/GW LC policy is based upon SM 20th 5210 B. (6).** If you analyze one GGA it must be acceptable or flag data. If you analyze more than one (do not have to analyze 3) the average of all GGAs must be 198 ± 30.5 mg/L (167.5-228.5 mg/L) or you flag the data.
65	What is the acceptance criterion of the CBOD GGA standard? [NC WW/GW LC policy based upon SM 20th 5210 B. (6) **or** NC WW/GW LC policy based upon guidance from EPA Region IV.			While the method states the CBOD GGA standard has same acceptance criterion as BOD, NC WW/GW LC policy, based upon EPA Region IV guidance, allows 164 ± 30.7 mg/L (133.3 – 194.7 mg/L). The lab must choose which acceptance criteria they will follow and not switch between

				them.
66	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (F).]			
67	Is a seeded blank analyzed? (Recommendation)			Not in methods – recommended – should approximate the final SCF
68	What is the average typical difference between the seeded blank and the calculated seed correction factor?			≈ ≤ 0.2 mg/L average difference between calculated FINAL seed correction factor and seeded blank is good. This serves as QC check of analyst technique and homogeneity of seeding material.
69	Are 5% of the samples set in duplicate? [15A NCAC 2H .0805 (a) (7) (C).]			Only have to duplicate one dilution to meet minimum requirement. If less than 20 samples per month analyzed must duplicate at least one sample per month.
70	Do duplicates check within 30% of each other? [NC WW/GW LC policy based upon SM 21st 5210 B. (7) (b).]			Duplicates having concentrations greater than the lower reporting limit of 2.0 mg/L must check within 30%. **See Flagging QC Failures document.**
71	Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [NC WW/GW LC policy based upon 15A NCAC 2B .0506 (b) (3) (J). and SM 21st 5210 B. (7) (b).]			**See Flagging QC Failures document.**
72	What is the reporting limit (PQL)? [SM 18th, 19th, 20th 5210 B. (6) (b).]			NC WW/GW LC policy is 2 mg/L based on SM 18th, 19th, 20th 5210 B. 6. b. **See Calculations and Reporting document.**
73	What is the most recent review/revision date of the SOP? [15A NCAC 2H .0805 (a) (7).]			

Additional Comments:

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Inspector: _____ Date: _____

Neutralizing Total Residual Chlorine in BOD Samples

It is acceptable to screen samples with DPD powder for the presence of Total Residual Chlorine (use pillows appropriate for the volume of sample tested). Generally total residual chlorine test strips are not adequate; however, these may be used for samples where interference with DPD precludes their use. If DPD yields no pink color, chlorine is absent. Document and proceed to set sample. If pink color is observed, chlorine is present. In those cases, the titration procedure outlined below must be used to determine the proper amount of Sodium Sulfite needed to neutralize chlorine in the sample.

Chemicals:

1. Sodium Sulfite solution - Dissolve 0.1575 g Na_2SO_3 in 100 mL distilled water. Prepare fresh daily.

2. 2% H_2SO_4 - Add 2 mL concentrated H_2SO_4 to 100 mL distilled water.

3. Potassium Iodide (KI) solution - Add 10 g KI to 100 mL distilled water. Initially this solution will be clear, but in a few days it will turn greenish yellow. That's ok.

4. Starch - Commercially available or see Standard Methods, 20th Edition - Method 4500-O C. (2) (d).

Procedure:

To 100 mL of sample, add approximately 1 mL of 2% H_2SO_4, 1 mL KI solution, and 1 mL starch.

If the solution remains clear, no chlorine is present. Document this on the bench sheet and proceed to set up the sample for BOD analysis.

If the solution turns blue, chlorine is present. Add the Sodium Sulfite solution, drop by drop, while stirring the sample, until the sample is clear again. Count the drops of Sodium Sulfite solution needed to neutralize the 100 mL sample. Add the relative volume of Sodium Sulfite solution to the volume of sample needed. For example, if it took 6 drops to neutralize the 100 mL sample volume and you need 300 mL of sample to set the dilutions you want, add 18 drops of Sodium Sulfite solution to 300 mL of sample. Document this on the bench sheet. Wait about 15 minutes and recheck the sample to verify the chlorine has been neutralized. Proceed to set up the sample.

Note: If the blue color returns after a few seconds, do not add more Sodium Sulfite solution. Recheck with DPD to verify that returning blue color is not caused by chlorine still in sample. If no chlorine is still present, add amount of Sodium Sulfite solution equivalent to when blue color first disappears. Adding an excessive amount of Sodium Sulfite solution creates an oxygen demand and will result in a false high BOD value.

Preparation of Synthetic Seed Material

Per the manufacturer's instructions:

1. Synthetic seed must be prepared using "buffered dilution water", that is laboratory water containing the required BOD nutrients. Seed is often incorrectly prepared using laboratory water without the nutrients added.

2. Seeding material should be both "stirred" and "aerated" during preparation. The extra oxygen from aeration appears to result in a more viable seeding material. This is accomplished by bubbling air through the mixture while stirring. (A common aquarium air pump and aeration stone may be used.)

3. Decant the supernatant to another clean container (after preparation), carefully leaving the bran behind. For the remainder of the test, gently stir and pipette aliquots to BOD bottles from the supernatant.

4. Stirring and aerating must occur for a minimum of 1 hour and seeding material must be used within 6 hours after preparation.

It is also recommended that heat be carefully applied, approximately 30 – 35 °C, during seed preparation. This may be helpful in producing a more viable bacteria population. Be careful not to overheat the solution. Excessive heat will inhibit or destroy the bacteria.

Calculations and Reporting

Seed Correction Factor (SCF) Calculations

Example: Seed controls of 20, 15, and 10 mLs are analyzed and 3 mLs seed is added to each bottle.

20 mL seed control yields: initial DO 8.0mg/L, final DO 4.4 mg/L, DO used = 3.6 mg/L

$$\frac{\text{20 mLs in SC}}{\text{3.6 mg/L DO used}} = \frac{\text{3 mLs seed /bottle}}{\text{x mg/L DO used}}$$

$20x = (3.6)(3)$

$$x = \frac{(3.6)(3)}{20}$$

$x = 0.54$ mg/L

15 mL seed control yields: initial DO 8.1mg/L, final DO 5.1 mg/L, DO used = 3.0 mg/L

$$\frac{\text{15 mLs in SC}}{\text{3.0 mg/L DO used}} = \frac{\text{3mLs seed /bottle}}{\text{x mg/L DO used}}$$

$15x = (3.0)(3)$

$$x = \frac{(3.0)(3)}{15}$$

$x = 0.60$ mg/L

10 mL seed control yields: initial DO 8.1mg/L, final DO 5.7 mg/L, DO used = 2.4 mg/L

$$\frac{\text{10 mLs in SC}}{\text{2.4 mg/L DO used}} = \frac{\text{3mLs seed /bottle}}{\text{x mg/L DO used}}$$

$10x = (2.4)(3)$

$$x = \frac{(2.4)(3)}{10}$$

$x = 0.72$ mg/L

Since all three dilutions met the depletion criterion of 2.0 mg/L DO used and the residual criterion of 1.0 mg/L DO remaining rule, all three **initial** seed correction factors (x) are averaged to obtain the **final** seed correction factor (SCF) as follows: $(0.54 + 0.60 + 0.72) \div 3 = 0.62$ mg/L.

The criterion to decide whether to use an initial seed correction factor in calculating the final seed correction factor is the 2 and 1 depletion rule, **not** whether the individual seed correction factor is between 0.6 and 1.0 mg/L. So, in this example, the 0.54 mg/L value is used since the dilution depleted at least 2.0 mg/L DO and had at least 1.0 mg/L DO remaining. Conversely, if a dilution does not meet the “2 and 1 rule” it is not used in calculating the final seed correction factor, even if the result is in the 0.6 - 1.0 mg/L range.

Sample Calculations and Reporting

For the following calculations, do not make corrections for the DO depletion by the dilution water blank (no blank subtractions).

Sample bottles that meet the "2 and 1 rule":

For each test bottle having ≥ 2.0 mg/L DO depletion and ≥ 1.0 mg/L residual DO ("2 and 1 rule"), calculate BOD as follows according to Standard Methods 21st Edition 5210 B. (7) (a):

$$BOD_5, \text{ mg/L} = \frac{(D_1 - D_2) - (S)\,V_s}{P}$$

D_1 = Initial DO, mg/L
D_2 = Final DO, mg/L
S = Oxygen uptake of seed per mL added to bottle,
S = 0 if sample is not seeded
V_s = Volume of seed added to bottle, mL
P = decimal volumetric fraction of sample,
1/p = dilution factor

A simpler equivalent formula:

BOD_5, mg/L = ((Initial DO) – (Final DO) – (Seed Correction Factor)) x (Dilution Factor)

Dilution Factor = 300／(volume of sample used)
Guidance for calculating the Seed Correction Factor is on the previous page.

Example Calculation #1

Initial DO	* Final DO	** DO used (Initial DO- Final DO)	Seed Correction Factor (SCF)	Corrected DO (Initial DO - Final DO - SCF)	Volume of Sample mL	Dilution Factor (300 / volume of sample used)	Calculated BOD_5 mg/L
8.0	6.9	1.1	0.8	0.3	6	50	15
8.0	6.1	1.9	0.8	1.1	30	10	11
8.0	4.1	3.9	0.8	3.1	75	4	**12.4**
8.0	1.5	6.5	0.8	5.7	150	2	**11.4**
8.0	0.13	7.87	0.8	7.07	300	1	>7.07

* Final DO must be ≥ 1.0 mg/L in order to meet the "2 and 1 rule". When reviewing data, all values in this column should be ≥ 1.0 mg/L. If not, dilutions should not be used in calculating final BOD value (unless no acceptable dilutions are obtained – see example #3 below).
** DO used (depleted) must be ≥ 2.0 mg/L in order to meet the "2 and 1 rule". When reviewing data, all values in this column should be ≥ 2.0 mg/L. If not, dilutions should not be used in calculating final BOD value (unless no acceptable dilutions are obtained – see example #2 below).

So, for the above data set, the first two dilutions (6 mL and 30 mL) do not meet the "2 and 1 rule" due to not depleting ≥ 2.0 mg/L, and the last dilution (300 mL) does not meet the "2 and 1 rule" due to having a final DO < 1.0 mg/L. Those values will not be factored into the reported value.

The reported value for the above data set would be 11.9 mg/L, which is the average of the two dilutions that meet the "2 and 1 rule" (12.4 mg/L and 11.4 mg/L).

Samples bottles that DO NOT meet the "2 and 1 rule":

<u>Example Calculation #2</u> – 100% Sample

If the DO depletion is less than 2.0 mg/L and the sample concentration is 100% (not diluted), use the above formula and report the value obtained and report the value as < 2 mg/L. (NC WW/GW LC policy is based on SM 18th, 19th and 20th Editions 5210 B. (6) (b)

Initial DO	Final DO	DO used (Initial DO- Final DO)	Seed Correction Factor (SCF)	Corrected DO (Initial DO - Final DO - SCF)	Volume of Sample mL	Dilution Factor (300 / volume of sample used)	Calculated BOD_5 mg/L
8.0	7.1	0.9	0.8	0.1	6	50	5.0
8.0	7.0	1.0	0.8	0.2	30	10	2.0
8.0	6.8	1.2	0.8	0.4	75	4	1.6
8.0	6.5	1.5	0.8	0.7	150	2	1.4
8.0	6.2	1.8	0.8	1.0	300	1	**1.0**

For the above data set, none of the dilutions met the "2 and 1 rule" because none used ≥ 2.0 mg/L DO. The data should be reported as < 2 mg/L.

<u>Example Calculation #3</u> – 2.0 mg/L Depletion not met

Initial DO	Final DO	DO used (Initial DO- Final DO)	Seed Correction Factor (SCF)	Corrected DO (Initial DO - Final DO - SCF)	Volume of Sample mL	Dilution Factor (300 / volume of sample used)	Calculated BOD_5 mg/L
8.00	7.10	0.90	0.80	0.10	1	300	30
8.00	7.00	1.10	0.80	0.30	2	150	45
8.00	6.60	1.40	0.80	0.60	5	60	36

For the above data set, none of the dilutions met the "2 and 1 rule" because none used ≥ 2.0 mg/L DO. This is because the sample dilutions set were too low. In this situation, some labs want to report the data as less than the value calculated if 2.0 mg/L had been used. In this example, that would be < 72 mg/L (that is, 2.0 mg/L DO used – 0.8 mg/L SCF = 1.2 mg/L X 60 dilution factor = 72 mg/L). NC WW/GW LC policy prohibits raising the (PQL) reporting limit because the "use 2 rule" was not met. Instead the value obtained is reported and flagged. This policy is designed to prevent labs from artificially raising the PQL by setting "too low" dilutions and not demonstrating permit compliance.

The reported value in this instance would be 36 mg/L (not < 72 mg/L). Additionally; the result would have to be qualified for not meeting the "2 and 1 rule". Intentionally setting low dilutions in order to artificially raise the PQL is unacceptable.

<u>Example Calculation #4</u> – Final DO < 1.0 mg/L

When all dilutions result in a final DO < 1.0 mg/L, select the bottle having the least amount of sample (greatest dilution) and use the above calculation. Report the result as greater than (>) the calculated value.

Initial DO	Final DO	DO used (Initial DO- Final DO)	Seed Correction Factor (SCF)	Corrected DO (Initial DO - Final DO - SCF)	Volume of Sample mL	Dilution Factor (300 / volume of sample used)	Calculated BOD_5 mg/L
8.00	0.35	7.65	0.80	6.85	6	50	**>342.5**
8.00	0.00	8.00	0.80	7.20	30	10	>72
8.00	0.00	8.00	0.80	7.20	75	4	>28.8
8.00	0.00	8.00	0.80	7.20	150	2	>14.4
8.00	0.00	8.00	0.80	7.20	300	1	>7.2

For the above data set, none of the dilutions met the “2 and 1 rule” because none had a final DO ≥ 1.0 mg/L. The reported value would be > 342.5 mg/L (subject to rounding) and the data must be qualified accordingly (see Flagging QC Failures document).

Flagging BOD Quality Control Failures

It is the intent of the State of North Carolina that all data generated for any permitted location under NPDES requirements is to be reported. "Every person subject to this section shall file certified monitoring reports setting forth the results of tests and measurements conducted pursuant to NPDES permit monitoring requirements." Reference: 15A NCAC 2B .0506 (a) (1). Additionally, "the results of all tests of the characteristics of the effluent, including but not limited to NPDES Permit Monitoring Requirements, shall be reported on monthly report forms." Reference: 15A NCAC 2B .0506 (b) (3) (J).

This means all data are reported and none are rejected by the laboratory or permittee. If all quality control requirements are not met, it is required that the data are flagged and the qualifications appear on the back of the Discharge Monitoring Report (DMR). Any rejection of data will be issued by the agency which receives the data.

NC WW/GW LC policy for flagging quality control failures is based upon the Quality Control requirements of the method as outlined in Standard Methods, 21st Edition – Method 5210 B. (7) (b) which states: "Identify results in the test reports when any of the following quality control parameters are not met:"

Anytime any of the following quality control failures occur the data must be flagged.

1. No sample dilutions deplete at least 2.0 mg/L DO and have a residual of at least 1.0 mg/L DO (unless 100% sample is analyzed).

2. The dilution water blank is greater than 0.20 mg/L.

3. The Glucose - Glutamic Acid (GGA) check falls outside the acceptance limits [i.e., 198 mg/L ± 30.5 mg/L (167.5 – 228.5 mg/L) or 164 ± 30.7 mg/L (133.3 – 194.7 mg/L) for CBOD]. When multiple GGA standards are analyzed, the average of those results must be within the acceptable limits.

4. Duplicates vary more than 30% between low and high values.

5. No seed control dilutions deplete at least 2.0 mg/L DO and have a residual of at least 1.0 mg/L DO.

The qualifying statement on the laboratory report form and/or the Discharge Monitoring Report (DMR) must state:

1. All QC requirements were not met, and

2. What the QC failure involved. For example, "blank was >0.20 mg/L", "GGA was less than 167.5 mg/L", "duplicates exceeded 30% difference due to low BOD concentration", etc.

It is recommended that the laboratory supervisor include a statement indicating whether the data is considered "valid", "questionable", or "invalid". This is a subjective decision based upon the severity of the QC failure and its impact on the value reported.

Data must always be reported. Accompanying documentation may be attached to justify any data believed to be invalid.

Luminescence DO (LDO) Daily Check

Although many manufacturers of LDO probes indicate that the probes require calibration only every 6 months or so, NC WW/GW LC requires that an LDO probe must be calibrated or have the calibration verified each day of use. Below is a procedure for verifying the calibration of an LDO probe.

1) Place probe in a plastic bag with a wet sponge or a BOD bottle partially filled with water
2) Make sure the bag is effectively sealed (zip, rubber band, or twist tie)
3) Allow appropriate instrument warm up time
4) Read D.O. and temperature
5) Check the reading vs. the Solubility Table below and apply appropriate atmospheric (barometric) pressure or altitude correction factor
6) Calculated D.O. value must verify meter reading within ± 0.5 mg/L (do NOT calculate and apply a correction factor to calculated D.O.).

Temp. °C	D.O. mg/L		Temp. °C	D.O. mg/L
4	13.11		19.5	9.18
4.5	12.94		20	9.09
5	12.77		20.5	9.00
5.5	12.61		21	8.92
6	12.45		21.5	8.83
6.5	12.30		22	8.74
7	12.14		22.5	8.66
7.5	11.99		23	8.58
8	11.84		23.5	8.50
8.5	11.70		24	8.42
9	11.56		24.5	8.34
9.5	11.42		25	8.26
10	11.29		25.5	8.18
10.5	11.16		26	8.11
11	11.03		26.5	8.04
11.5	10.90		27	7.97
12	10.78		27.5	7.90
12.5	10.66		28	7.83
13	10.54		28.5	7.76
13.5	10.42		29	7.69
14	10.31		29.5	7.62
14.5	10.20		30	7.56
15	10.08		30.5	7.50
15.5	9.98		31	7.43
16	9.87		31.5	7.37
16.5	9.77		32	7.31
17	9.67		32.5	7.24
17.5	9.57		33	7.18
18	9.47		33.5	7.12
18.5	9.38		34	7.07
19	9.28		34.5	7.01

Atmospheric Pressure mm Hg	Equivalent Altitude Ft.	Correction Factor
760	0	1.00
752	278	.99
745	558	.98
737	841	.97
730	1126	.96
722	1413	.95
714	1703	.94
707	1995	.93
699	2290	.92
692	2587	.91
684	2887	.90
676	3190	.89
669	3496	.88
661	3804	.87
654	4115	.86
646	4430	.85
638	4747	.84
631	5067	.83
623	5391	.82
616	5717	.81
608	6047	.80
600	6381	.79
593	6717	.78

Ref: YSI Model 5000/5100 DO Meter Manual. Slight variations in DO, pressure, and/or altitude may be found in other manuals.

Example: If ambient temperature is 21°C and elevation is approximately 1126 ft, the theoretical DO would be:

8.92 X 0.96 = 8.56 mg/L

or, If ambient temperature is 21°C and the atmospheric (barometric) pressure is 745 mm Hg, the theoretical DO would be:

8.92 X 0.98 = 8.74 mg/L