

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: **BIOCHEMICAL OXYGEN DEMAND (BOD₅/CBOD₅)**
 Method: **Standard Methods 5210 B - 2016**

BOD is considered a method-defined parameter per the definition in the Code of Federal Regulations, Part 136.6, Section (a) (5). This means that the method may not be modified per Part 136.6, Section (b) (3).

BOD - SM 5210 B-2016 (Aqueous)	CBOD - SM 5210 B-2016 (Aqueous)
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EQUIPMENT:

Incubation Bottles, 60 mL or 300 mL	DO Meter – model:	Membrane Electrode
Air incubator or water bath	pH meter – model:	LDO Electrode
Graduated cylinders	Wide tipped pipettes	

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	L A B	S O P	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 review/revision dates and procedural edits 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 SOP documents.
3	Is there North Carolina data available for review?			If not, review PT data
	PRESERVATION and STORAGE	L A B	S O P	EXPLANATION
4	Are samples iced to above freezing but ≤ 6 °C during shipment? [40 CFR 136.3 Table II]			40 CFR footnote 2 allows 15 minutes for sample preservation, including thermal. This means that if a sample is received in the lab within 15 minutes it is not required to be on ice. Document temperature downward trend for short transport samples.
5	Are samples refrigerated to above freezing but ≤ 6 °C during storage? [40 CFR 136.3 Table II]			
6	Are samples analyzed within 48 hours of collection? [40 CFR 136.3 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	L A B	S O P	EXPLANATION
7	Is the pH meter calibrated? [15A NCAC 02H .0805 (a) (7)]			Calibrated according to manufacturer's instructions.
8	Is the calibration of the pH meter documented? This is considered pertinent information. [15A NCAC 02H .0805 (a) (7) (E)]			All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
9	How is the DO meter calibrated? [SM 5210 B-2016 (5) (g)] Answer:			References DO 4500-O G. for membrane electrode. References DO 4500-O H for optical probe. Calibrate DO/LDO meter per manufacturer's instructions. Generally DO will be saturated air calibration based upon temperature, elevation and/or barometric pressure. LDO probe must have calibration verified each day of use if

				meter does not allow calibration. 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.
10	Is the DO meter calibration documented on day one and day five? This is considered pertinent information. [15A NCAC 02H .0805 (a) (7) (E)]			All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
11	Is the DO meter calibration checked for drift during and at the end of the sample set? [SM 5210 B-2016 (5) (g)] [NC WW/GW LCB BOD ₅ /CBOD ₅ – DO Meter Drift Check Policy]			A drift check is required at the end of each sample set, however, drift checks throughout the sample run are recommended for large sample sets since all bottles must be reanalyzed since calibration (or last acceptable) drift check if the check is unacceptable. Make sure drift checks are performed on bottle filled with water – not the calibration bottle which is partially full – D.O. not stable enough in air
12	Is the meter calibration drift check documented? [NC WW/GW LCB BOD ₅ /CBOD ₅ – DO Meter Drift Check Policy]			Immediately after calibration, measure the DO of a BOD bottle of dilution water. Stopper the bottle. Document the concentration on the benchsheet (day one initial drift check). At the end of the sample set (i.e., a group of samples analyzed without interruption) reanalyze the drift check bottle and document the concentration (day one final drift check).
13	What is the acceptance criterion for the drift check? [NC WW/GW LCB BOD ₅ /CBOD ₅ – DO Meter Drift Check Policy] Answer:			Must agree within ± 0.2 mg/L.
14	What corrective action is taken if the drift check is outside the acceptance criterion? [NC WW/GW LCB BOD ₅ /CBOD ₅ – DO Meter Drift Check Policy] [15A NCAC 2H .0805 (a) (7) (B)] Answer:			If the meter drifts more than 0.2 mg/L, recalibrate the meter and reanalyze all samples since the last acceptable drift check. If additional samples are analyzed later the same day, begin the set with a drift check and compare to the initial drift check value. If the drift check fails, recalibrate prior to starting the additional sample set and begin new drift checks for that set.
	PROCEDURE – Dilution Water	L	S	EXPLANATION
		A	O	
		B	P	
15	What is the source of dilution water? [SM 5210 B-2016 (4) (c)] Answer:			Obtain water from suitable source (i.e., distilled, tap, or reagent-grade water). Make sure water is free of heavy metals, specifically copper (<0.05 mg/L) and toxic substances [e.g., chlorine (<0.10 mg/L)] that can interfere with BOD measurements. Protect source-water quality by using clean glassware, tubing, and bottles. Deionized (DI) water often contains enough organics and microorganisms to cause the dilution-water QC check to fail (5210 B.6c). DI water is not recommended unless dilution-water blanks consistently meet QC limits.
16	Is dilution water aged / conditioned? [SM 5210 B-2016 (4) (c)]			Ageing water may improve blanks. Do whatever works for that lab. Recommend not adding nutrients to water until ready to use (no more than 24 hours prior to use).
17	If so, how and for how long? [SM 5210 B-2016 (4) (c)] Answer:			Recommend storing water at least overnight in an incubator to allow temperature and DO to stabilize.
18	Is dilution water and container free of biological growth? [SM 5210 B-2016 (4) (c)]			Storage may improve the quality of some source waters but may allow biological growth to deteriorate others.
19	Is the dilution water approximately room temperature before use? [SM 5210 B-2016 (5) (a)]			SM states: Check to ensure the dissolved oxygen concentration is at least 7.5 mg/L before using water for BOD tests. Mix thoroughly and bring temperature to 20 ± 3 °C. Temperature does not have to be documented – see explanation for question #26 below.
20	Are the nutrient solutions prepared or purchased? [SM 5210 B-2016 (3)] Answer:			Phosphate Buffer, Magnesium Sulfate, Calcium Chloride, Ferric Chloride

21	If purchased – pillows or individual bottles? [SM 5210 B-2016 (3)] Answer:			
22	Is the phosphate buffer (prepared or purchased) documented to be pH 7.2? [SM 5210 B-2016 (3) (a)]			Purchased will be documented to be 7.2 by manufacturer. Method allows two ways to prepare, one that should be 7.2 as prepared and another that may be adjusted to 7.2 if needed.
23	Are nutrients within manufacturer or laboratory assigned expiration dates? [15A NCAC 02H .0805 (a) (7) (K)]			Chemicals and reagents exceeding the expiration date shall not be used.
24	Are nutrients free of precipitation or biological growth? [SM 5210 B-2016 (3)]			Liquid reagents are recommended to be stored in a refrigerator.
25	Are nutrients added at rate of 1 mL each per liter of dilution water? [SM 5210 B-2016 (5) (a)]			
	PROCEDURE – Sample Preparation	L A B	S O P	EXPLANATION
26	Are samples warmed to approximately room temperature? [SM 5210 B-2016 (5) (b)]			SM states: Bring samples to 20 ± 3 °C before making dilutions. It is OK to make the assumption that if initial DOs are acceptable – that is 7.0-9.0 mg/L for samples that sample temperature was OK. The acceptable DO in the BOD is a function of sample volume used and initial DO of dilution water – if using small sample volume the temperature is not critical once it is mixed with dilution water – if using larger sample volume and initial DO is acceptable it is obvious temperature was OK – the entire reason for a temperature range for samples it to obtain required initial DO range – if that is OK we have never required actually measuring sample temperature nor documenting sample temperature.
27	Is each sample pH checked and documented before analysis? [SM 5210 B-2016 (4) (b) (1)]			
28	If sample pH is not 6.0-8.0 S.U., is it adjusted to between 6.5 and 7.5 S.U.? [SM 5210 B-2016 (4) (b) (1)]			
29	Is the pH adjustment performed so that it does not dilute the sample more than 0.5%? [SM 5210 B-2016 (4) (a) (1)]			
30	Is each pH adjustment 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.
31	Are all samples checked for the presence of residual chlorine? [SM 5210 B-2016 (4) (b) (2)]			O-R (Oxidizing/Reducing agents) testing is not in the method. Adding Potassium Bi-iodate (not listed in method with chemicals used in test) to neutralize reducing agents is old practice. Is not in method – must not be done. This is especially true with widespread 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.
32	How are the samples checked for chlorine? [SM 5210 B-2016 (4) (b) (2)] Answer:			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. Ultra-low TRC test strips may be used. If using test strips, it is recommended the maximum allowable chlorine concentration be 0.1 mg/L.
33	If chlorine is present, is it properly neutralized with sodium sulfite? [SM 5210 B-2016 (4) (b) (2)]			See Neutralizing TRC in BOD Samples document.
34	Is this documented? This is considered pertinent information. [15A NCAC 02H .0805 (a) (7) (E)]			The amount (usually expressed as drops per 100 mL) of sodium sulfite must be documented.

35	Is sodium sulfite prepared each day it is used? [SM 5210 B-2016 (3) (f)]			Method says prepare daily. 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. Excess sodium sulfite will create an oxygen demand.
36	Is Hydrogen Peroxide (H ₂ O ₂) present in any samples? [SM 5210 B-2016 (4) (b) (5)]			
37	If so, how is hydrogen peroxide treated? [SM 5210 B-2016 (4) (b) (5)] Answer:			H ₂ O ₂ can cause supersaturated DOs. May see higher DO after 5 days than initial DO. Treatment – Mix samples vigorously in open container until H ₂ O ₂ dissipates. Check adequacy of H ₂ O 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 – particularly from industries using bleaching processes such as textile plants, paper mills and industrial laundries.
	PROCEDURE – Seeding	L A B	S O P	EXPLANATION
38	Are samples seeded if required? [SM 5210 B-2016 (4) (d)]			Samples needing seeding: pH outside acceptable range, disinfected (UV, chlorine, chlorinated/dechlorinated etc.), industrial, high-temperature, wastes stored more than 6 hours after collection . May just seed all samples - that's OK.
	Disinfected wastes?			
	Wastes having pH values less than 6 or greater than 8?			
	Wastes stored more than 6 hours after collection?			
39	What is the source of the seeding material? [SM 5210 B-2016 (4) (d)] Answer:			Can use commercial seed, influent from domestic source, receiving waters.
40	If commercial seed (e.g., Polyseed®, BioSeed®, etc.) is used, how is it prepared? [Manufacturer's instructions] Answer:			See Preparation of Synthetic Seed Material document .
41	If commercial seed is not used, how is it prepared? SM 5210 B-2016 (4) (d)] Answer:			Suitable sources: use supernatant from settled domestic wastewater, effluent from primary clarifiers, diluted mixed liquor from an aeration basin, undisinfected effluent, or receiving water below the point of discharge Do not use effluent or mixed liquor unless nitrifying inhibitors are used – that is CBOD analyzed. Do not use effluent that has been disinfected.
42	Is the seed agitated or stirred during transfer to ensure homogeneity? [SM 5210 B-2016 (5) (d)]			
43	How many seed controls are analyzed? [SM 5210 B-2016 (6) (d)] Answer:			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. (6)(d) states IDEALLY make 3 dilutions.
44	Is the seed correction calculated correctly from the seed controls? [SM 5210 B-2016 (7) (a) (1)]			See Calculations and Reporting document .
45	Is the criterion of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L used to determine which bottles to use for the seed correction calculation? [SM 5210 B-2016 (7) (a) (1)]			
46	Are the results of all acceptable seed control analyses averaged? [SM 5210 B-2016 (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. 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; check or change the seed source

	PROCEDURE- CBOD ₅	L A B	S O P	EXPLANATION
47	If CBOD ₅ is required, is a nitrification inhibitor added to all samples, seed controls and GGAs? [SM 5210 B-2016 (5) (e) and (6) (c)]			The seed controls and GGAs with nitrification inhibition are only used for CBOD samples and are set up in addition to non-inhibited seed controls and GGAs if both BOD and CBOD are analyzed. Nitrification inhibitor must NOT be added to blank per SM 5210 B-2016 (6) (c).
48	If BOD/CBOD samples are analyzed together, are separate seed controls and GGAs set up? [SM 5210 B-2016 (5) (e) and (6) (d)]			
49	What nitrification inhibitor is used for CBOD ₅ samples? [SM 5210 B-2016 (5) (e)] Answer:			TCMP (2-chloro-6-trichloromethyl pyridine) and ATU (allylthiourea solution) are allowed. TCMP is preferred. 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).
50	If TCMP (2-chloro-6-trichloromethyl pyridine) is used, is it added at a rate of 3 mg/300 mL bottle when the bottle is at least 2/3 full of diluted sample and mixed well? [SM 5210 B-2016 (5) (e) (1)]			
51	If ATU (allylthiourea) is used, is it added at a rate of 0.3 mL/300 mL bottle when the bottle is at least 2/3 full of diluted sample and mixed well? [SM 5210 B-2016 (5) (e) (2)]			
52	Is the ATU prepared fresh every two weeks? [SM 5210 B-2016 (3) (g) (2)]			
53	All questions on checklist apply to CBOD – the above are just additional questions exclusive to CBOD			
	PROCEDURE- Sample Analysis	L A B	S O P	EXPLANATION
54	Is the initial DO of the blank at least 7.5 mg/L? [SM 5210 B-2016 (5) (a)]			Initial DO of blank must be at least 7.5 mg/L
55	If not, what corrective action is taken? [15A NCAC 02H .0805 (a) (7) (B)] Answer:			If not, add DO by shaking bottle or by aerating with organic free filtered air. Alternately store the water in cotton plugged bottles long enough for the DO concentration to approach saturation.
56	Is the initial DO of all bottles other than blank between 7 and 9 mg/L? [SM 5210 B-2016 (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 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.
57	If not, what corrective action is taken? [15A NCAC 02H .0805 (a) (7) (B)] Answer:			Need to warm up samples and agitate vigorously to reduce DO. Aerate or cool samples to raise DO. Acceptable temperature range is 17-23 °C.
58	Do documented times demonstrate that initial DOs are measured within 30 minutes of preparing dilutions? [SM 5210 B-2016 (5) (g)]			
59	Is the sample stirred during DO measurement or mixed immediately prior to measurement? [SM 5210 B-2016 (5) (f)]			Membrane probes require flow across the membrane. LDO type probes do not.
60	Are at least three dilutions set for samples? [SM 5210 B-2016 (5) (c)]			Make at least three dilutions estimated to meet the 2-1 rule. More dilutions are recommended for unfamiliar samples. Two dilutions are allowed if experience with a particular sample source produces at least one bottle with acceptable minimum DO depletions and residual limits (5210 B.6a).
61	How are sample volumes measured? [SM 5210 B-2016 (5) (c)] Answer:			Using a wide-tipped pipet or graduated cylinder, add the desired amount of prepared sample to individual volumetric cylinders or flasks. Mix the sample well immediately before pipetting to avoid solids loss caused by settling. For dilutions greater than 1:300 make a primary dilution before making final dilution. This would be

				any measurements of less than 1 mLs sample volume if diluting directly into BOD bottles.
62	For dilutions with >67% sample (> 201 mL), are extra nutrients added? [SM 5210 B-2016 (5) (c) (2)]			Easiest 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. Add at a rate of 0.30 mL/300 mL bottle.
63	Is this documented? This is considered pertinent information. [15A NCAC 02H .0805 (a) (7) (E)]			
64	If the azide modification of the iodometric method (SM 4500 O C) is used to measure DO, is an additional bottle prepared for each dilution to measure the initial DO? [SM 5210 B-2016 (5) (g)]			
65	Are the samples incubated for five days ± 6 hours? [SM 5210 B-2016 (5) (h) and (i)]			
66	Are samples incubated in the dark at 20 ± 1 °C? [SM 5210 B-2016 (5) (h)]			
67	Are bottles completely filled and stoppered in such a manner that leaves no bubbles in the bottle? [SM 5210 B-2016 (5) (f)]			
68	Are water seals maintained on the bottles during incubation? [SM 5210 B-2016 (5) (f)]			Obtain satisfactory water seals by adding water to the flared mouth of BOD bottles. Make sure caps are used on bottles to prevent evaporation. Alternatively, may be incubated in water bath. If water seals are not maintained air bubbles may form in bottles.
69	Are all dilutions that deplete at least 2.0 mg/L DO and have at least 1.0 mg/L DO remaining, averaged for final BOD results? [SM 5210 B-2016 (7) (b)]			Evaluate whether dilutions meet the depletion requirement prior to applying the seed correction factor.
70	Are seed corrections properly subtracted from the seeded samples? [SM 5210 B-2016 (7) (a) (1)]			See Calculations and Reporting document.
71	Is the final BOD of the samples properly calculated? [SM 5210 B-2016 (7) (a) (1)]			See Calculations and Reporting document.
	QUALITY ASSURANCE	L A B	S O P	EXPLANATION
72	What is the reporting limit (PQL)? [NC WW/GW LCB BOD ₅ /CBOD ₅ Reporting Limit Policy] Answer:			Only bottles giving a minimum Dissolved Oxygen (DO) depletion of 2.0 mg/L and a residual DO of 1.0 mg/L after 5 days of incubation are considered to produce valid data. It is not acceptable to elevate the reporting limit and report as less than the value that would have been obtained if the dilution had depleted the required 2.0 mg/L. It is required that the data be flagged as a quality control failure if it does not deplete the 2.0 mg/L. The only exception to this is when a 100% sample does not deplete 2.0 mg/L. In this case, report the results without qualification as calculated or as <2.0 mg/L. The laboratory must be consistent in applying whichever option is chosen and this must be clearly outlined in the SOP. See Calculations and Reporting document.
73	Is the date/time samples are placed into the incubator documented? [15A NCAC 02H .0805 (a) (7) (F)]			The date and time that samples are placed into and removed from ovens, water baths, incubators and other equipment shall be documented if a time limit is required by the method.
74	Is the date/time samples are taken out of the incubator documented? [15A NCAC 02H .0805 (a) (7) (F)]			The date and time that samples are placed into and removed from ovens, water baths, incubators and other equipment shall be documented if a time limit is required by the method.
75	Are incubator temperatures documented? [15A NCAC 02H .0805 (a) (7) (I)]			Each day samples are placed into or removed from an incubator, oven, water bath, refrigerator, or other temperature-controlled device, the temperature shall be checked, recorded, dated, and initialed. Must be 20 ± 1 °C
76	Are at least two dilution water blanks analyzed for each batch of dilution water? [SM 5210 B-2016 (6) (c)]			If more than one container of water is used, a minimum of 2 blanks must be analyzed for each container of water.
77	Is the average DO depletion of the dilution water blank ≤ 0.2 mg/L? [SM 5210 B-2016 (6) (c)]			Preferably < 0.1 mg/L. Blank readings must be made to two decimal places. An exception may be with analog

			<p>meters which are not this sensitive. It is recommended that these be replaced when budgets allow. If the lab would like something in writing to help justify getting a new meter, we can point to this.</p> <p>Sample results associated with blanks reading >0.2 mg/L must be qualified. If multiple containers of water are used, only the samples associated with unacceptable blank values must be qualified, not samples associated with acceptable blank values. This means samples associated with the different blanks must be tracked by the laboratory. The average of the blanks for each container must be ≤ 0.2 mg/L.</p> <p>See Flagging QC Failures document.</p>
78	<p>If not, what corrective action is taken? [15A NCAC 02H .0805 (a) (7) (B)]</p> <p>Answer:</p>		<p>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.</p>
79	[Optional] Is a seeded blank analyzed? (Recommendation)		Not required. Should approximate the final SCF.
80	<p>What is the average typical difference between the seeded blank and the calculated seed correction factor?</p> <p>Answer:</p>		<p>$\approx \leq 0.2$ 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. Check the depletion (mg/L of DO) of the seeded blank and compare that value to the calculated value of the FINAL seed correction (value subtracted from each sample). Values should be on average within about 0.2 mg/L of each other.</p>
81	Are three bottles of glucose-glutamic acid standard (GGA) analyzed with each set of samples? [SM 5210 B-2016 (6) (b)]		
82	<p>What is the source of the GGA standard? [SM 5210 B-2016 (3) (h) and (6) (b)]</p> <p>Answer:</p>		May make or buy prepared. If making, dry reagents at 103 °C for 1 hr.
83	<p>How frequently is the GGA standard made? [SM 5210 B-2016 (3) (h)]</p> <p>Answer:</p>		<p>May be held if maintained in sterile conditions and stored at ≤ 6 °C. 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 mL into sample bottle then discard any excess. Should not try to hold longer than approximately 1 month. Discard solutions if evidence of contamination is indicated (i.e., growth occurs in the stock bottle or GGA test results are consistently low.)</p>
84	Is the GGA standard analyzed at a 2% dilution? [SM 5210 B-2016 (6) (b)]		<p>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. Instructions from vendor state to set 6 mLs and divide answer obtained by 2. Cannot do that per EPA Region IV. OK to use if diluted by half prior to dispensing into BOD bottle. For example, use 5 mLs of GGA and add 5 mLs of lab water (not buffered BOD water) then pipet 6 mLs of that solution into BOD bottle.</p>
85	Is the GGA standard seeded? [SM 5210 B-2016 (5) (d)]		Never have only GGA and seed in bottle. Always have some dilution water in bottle before adding GGA and seed. Bacteria in seed will begin to consume sugars (glucose) immediately.
86	<p>What is the acceptance criterion for the average of the three GGA bottles? [SM 5210 B-2016 (8) (a) and 40 CFR 136.3 Table IB, Footnote 85]</p> <p>BOD:</p>		<p>SM 5210 B-2016 (8) and 40 CFR 136.3 Table IB, Footnote 85 state that the lab may use control charts to determine the acceptance range of GGA standards, however, the lab's criterion must not be wider than 198 ± 30.5 mg/L for BOD. The acceptance criterion for CBOD may be wider based on control charts.</p>

	CBOD:		<p>If the laboratory has determined the GGA acceptance criterion through control chart, were the following requirements met?</p> <ul style="list-style-type: none"> • Dissolved oxygen uptake from the seed contribution is between 0.6–1.0 mg/L. • Control charts are performed on at least 25 GGA checks with three standard deviations from the derived mean. • The RSD must not exceed 7.5%. • Any single GGA value cannot be less than 150 mg/L or higher than 250 mg/L.
87	If the GGA check is not acceptable, what corrective action is taken? [15A NCAC 02H .0805 (a) (7) (B)] Answer:		<p>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.</p>
88	Is a duplicate analyzed daily or with each batch of 20 or fewer samples? [SM 5020 B-2017 Table 5020:I, Footnote 2.]		<p>Only have to duplicate one dilution of the three set up to meet minimum requirement.</p>
89	What is the acceptance criterion for duplicates? [15A NCAC 02H .0805 (a) (7)] Answer:		<p>Laboratories must set their own acceptance criterion. Duplicates having concentrations greater than the lower reporting limit of 2.0 mg/L should check within 30% since that is the criterion for the difference between the high and low calculated values from dilutions of the same sample. See NC WW/GW LCB Policy for Flagging QC Failures document.</p>
90	Are calculated results for replicates within 30% of high and low values? [SM 5210 B-2016 (7) (b)]		<p>Samples with large difference between the computed BOD for different dilutions (e.g., the highest value is >30% larger than the lowest value) may indicate a toxic substance or analytical problems. When the effect becomes repetitive, investigate to identify the cause.</p>
91	If not, what corrective action is taken? [15A NCAC 02H .0805 (a) (7) (B)] Answer:		
92	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 (a) (7) (B)]		<p>See NC WW/GW LCB Policy for Flagging QC Failures document.</p> <p>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.</p>

Additional Comments:

Inspector: _____ Date: _____

Neutralization of Total Residual Chlorine in BOD₅/CBOD₅ Samples Policy

(NC WW/GW LCB 08/06/2021)

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). If pink color is observed, chlorine is present. Ultra-low type Total Residual Chlorine test strips capable of detecting ≤ 0.1 mg/L may also be used. If chlorine is present, the titration procedure outlined below must be used to determine the proper amount of Sodium Sulfite needed to neutralize chlorine in the sample. If chlorine is absent, document and proceed to set sample.

Chemicals:

1. Sodium Sulfite solution - Dissolve 0.1575 g Na₂SO₃ in 100 mL distilled water. Prepare fresh daily.
2. 2% H₂SO₄ - Add 2 mL concentrated H₂SO₄ to 100 mL distilled water. 10 mL of 1:1 acetic acid may be substituted for the H₂SO₄ solution.
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 use either an aqueous solution or soluble starch powder mixture. To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid (as a preservative) in 100 mL hot distilled water.

Procedure:

To 100 mL of sample, add approximately 1 mL of 2% H₂SO₄, 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 10 to 20 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 8.0 mg/L, final DO 4.4 mg/L, DO used = 3.6 mg/L

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

$$20x = (3.6) (3)$$

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

$$x = 0.54 \text{ mg/L}$$

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

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

$$15x = (3.0) (3)$$

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

$$x = 0.60 \text{ mg/L}$$

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

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

$$10x = (2.4) (3)$$

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

$$x = 0.72 \text{ mg/L}$$

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: $(0.54 + 0.60 + 0.72) \div 3 = 0.62 \text{ mg/L}$.

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.

Final SCF values may be rounded to one decimal place.

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” prior to applying the seed correction factor:

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 SM 5210 B-2016 (7) (a):

$$\text{BOD}_5, \text{ mg/L} = \frac{(D_1 - D_2) - (S) V_s}{P}$$

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:

$$\text{BOD}_5, \text{ mg/L} = ((\text{Initial DO}) - (\text{Final DO}) - (\text{Seed Correction Factor})) \times (\text{Dilution Factor})$$

Dilution Factor = $300 / (\text{volume of sample used})$

Guidance for calculating the Seed Correction Factor is on the previous page.

Example Calculation #1

D_1 (mg/L)	* D_2 (mg/L)	** DO used ($D_1 - D_2$) (mg/L)	Seed Correction Factor (SCF) (mg/L)	Corrected DO ($D_1 - D_2 - \text{SCF}$) (mg/L)	Sample Volume (mL)	Dilution Factor ($300 / \text{Sample}$ Volume)	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).

Sample bottles that DO NOT meet the “2 and 1 rule” prior to applying the seed correction factor:**Example Calculation #2 – 100% Sample**

D ₁ (mg/L)	D ₂ (mg/L)	DO used (D ₁ -D ₂) (mg/L)	Seed Correction Factor (SCF) (mg/L)	Corrected DO (D ₁ - D ₂ - SCF) (mg/L)	Sample Volume (mL)	Dilution Factor (300 / Sample Volume)	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 is reported without qualification as 1.0 mg/L or < 2.0 mg/L. [NC WW/GW LCB Reporting Limit Policy]

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

D ₁ (mg/L)	D ₂ (mg/L)	DO used (D ₁ -D ₂) (mg/L)	Seed Correction Factor (SCF) (mg/L)	Corrected DO (D ₁ - D ₂ - SCF) (mg/L)	Sample Volume (mL)	Dilution Factor (300 / Sample Volume)	Calculated BOD ₅ (mg/L)
8.0	7.1	0.9	0.8	0.1	1	300	30
8.0	7.0	1.1	0.8	0.3	2	150	45
8.0	6.6	1.4	0.8	0.6	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 LCB Reporting Limit 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. [NC WW/GW LCB BOD₅/CBOD₅ Reporting Limit and Quality Control Failure Flagging Policies].

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

D ₁ (mg/L)	D ₂ (mg/L)	DO used (D ₁ -D ₂) (mg/L)	Seed Correction Factor (SCF) (mg/L)	Corrected DO (D ₁ - D ₂ - SCF) (mg/L)	Sample Volume (mL)	Dilution Factor (300 / Sample Volume)	Calculated BOD ₅ (mg/L)
8.0	0.35	7.65	0.8	6.85	6	50	>342.5
8.0	0.00	8.00	0.8	7.20	30	10	>72
8.0	0.00	8.00	0.8	7.20	75	4	>28.8
8.0	0.00	8.00	0.8	7.20	150	2	>14.4
8.0	0.00	8.00	0.8	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 [NC WW/GW LCB BOD₅/CBOD₅ Quality Control Failure Flagging Policy].

Occasionally, depletions for your dilution scheme will not meet any of the above examples. If that occurs, please contact the NC WW/GW LCB office for advice on how to calculate and report on a case-by-case basis.

BOD₅/CBOD₅ Quality Control Failure Flagging Policy
(NC WW/GW LCB 08/06/2021)

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. All dilutions result in a residual DO <1.0 mg/L.
3. The average DO depletion of dilution water blanks is greater than 0.2 mg/L.
4. The average of the three Glucose - Glutamic Acid (GGA) check standards falls outside the acceptance limits [i.e., 198 mg/L ± 30.5 mg/L (167.5 – 228.5 mg/L) or as determined by control chart prescribed in Footnote 85 of Table IB in 40 CFR Part 136.3, July 19, 2021]
5. Duplicate dilutions of the same sample vary more than the laboratory established acceptance limit.
6. Valid high and low (i.e., from different dilutions) calculated values of the same sample vary by more than 30%.
7. 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 DMR must state:

1. All QC requirements were not met, and;
2. What QC failures were involved. For example, “blank average value was >0.2 mg/L”, “GGA was less than 167.5 mg/L”, “duplicates exceeded the acceptance criterion 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 questionable or 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 LCB 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	Atmospheric Pressure mm Hg	Equivalent Altitude Ft.	Correction Factor
4	13.11	19.5	9.18	760	0	1.00
4.5	12.94	20	9.09	752	278	.99
5	12.77	20.5	9.00	745	558	.98
5.5	12.61	21	8.92	737	841	.97
6	12.45	21.5	8.83	730	1126	.96
6.5	12.30	22	8.74	722	1413	.95
7	12.14	22.5	8.66	714	1703	.94
7.5	11.99	23	8.58	707	1995	.93
8	11.84	23.5	8.50	699	2290	.92
8.5	11.70	24	8.42	692	2587	.91
9	11.56	24.5	8.34	684	2887	.90
9.5	11.42	25	8.26	676	3190	.89
10	11.29	25.5	8.18	669	3496	.88
10.5	11.16	26	8.11	661	3804	.87
11	11.03	26.5	8.04	654	4115	.86
11.5	10.90	27	7.97	646	4430	.85
12	10.78	27.5	7.90	638	4747	.84
12.5	10.66	28	7.83	631	5067	.83
13	10.54	28.5	7.76	623	5391	.82
13.5	10.42	29	7.69	616	5717	.81
14	10.31	29.5	7.62	608	6047	.80
14.5	10.20	30	7.56	600	6381	.79
15	10.08	30.5	7.50	593	6717	.78
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			

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:

$$8.92 \times 0.96 = 8.56 \text{ mg/L}$$

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

$$8.92 \times 0.98 = 8.74 \text{ mg/L}$$