

## NC DENR/DWQ WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION

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

Parameter: **BIOCHEMICAL OXYGEN DEMAND (BOD<sub>5</sub>/CBOD<sub>5</sub>)**  
Method: **Standard Methods 5210 B - 2001**

BOD - SM 5210 B-2011 (Aqueous)	CBOD - SM 5210 B-2011 (Aqueous)
BOD - SM 5210 B (ASTM D888-05 LDO) (Aqueous)	CBOD - SM 5210 B (ASTM D888-05 LDO) (Aqueous)
BOD - SM 5210 B (Hach 10360 LDO) (Aqueous)	CBOD - SM 5210 B (Hach 10360 LDO) (Aqueous)

## 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 2H .0805 (a) (7)]  <b>Date:</b>			Quality assurance, quality control, and Standard Operating Procedure documentation shall indicate the effective date of the document and be reviewed every two years and updated if changes in procedures are made. Verify proper method reference. During review notate deviations from the approved method and SOP.
2	Are all revision dates and actions tracked and documented? [15A NCAC 2H .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 2H .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 2H .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-2011 (5) (g)-2011] <b>ANSWER:</b>			References DO 4500-O G. for electrode. 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). <b>See attached <a href="#">LDO Daily Check handout</a>.</b>
10	Is the DO meter calibration documented on day one and day five? This is considered pertinent information. [15A NCAC 2H .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-2011 (5) (g)]			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? This is considered pertinent information. [15A NCAC 2H .0805 (a) (7) (E)]			All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
13	Do the drift checks agree within $\pm 0.2$ mg/L? [NC WW/GW LC policy]			
14	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (B)] <b>ANSWER:</b>			Meter must be recalibrated. All bottles read since the last acceptable drift check (or calibration if no other checks were performed) must be reread.
	<b>PROCEDURE – Dilution Water</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
15	What is the source of dilution water? [SM 5210 B-2011 (4) (c)] <b>ANSWER:</b>			Use demineralized, distilled, tap, or natural water. Basically any water source is OK as long as QC is met (no chlorine, metals, etc.).
16	Is dilution water aged / conditioned? [SM 5210 B-2011 (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-2011 (4) (c)] <b>ANSWER:</b>			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-2011 (4) (c)]			
19	Is the dilution water approximately room temperature before use? [SM 5210 B-2011 (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 $20 \pm 3$ °C.  Temperature does not have to be documented – see explanation for question #25 below.
20	Are the nutrient solutions prepared or purchased? [SM 5210 B-2011 (3)] <b>ANSWER:</b>			Phosphate Buffer, Magnesium Sulfate, Calcium Chloride, Ferric Chloride
21	If purchased – pillows or individual bottles? [SM 5210 B-2011 (3)] <b>ANSWER:</b>			

22	Is the phosphate buffer (prepared or purchased) documented to be pH 7.2? [SM 5210 B-2011 (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 2H .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-2011 (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-2011 (5) (a)]			
	<b>PROCEDURE – Sample Preparation</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
26	Are samples warmed to approximately room temperature? [SM 5210 B-2011 (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 and 7.5-9.0 for the blank 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-2011 (4) (b) (1)]			
28	If sample pH is not 6.0-8.0, is it adjusted to 7.0-7.2? [SM 5210 B-2011 (4) (b) (1)]			
29	Is the pH adjustment performed so that it does not dilute the sample more than 0.5%? [SM 5210 B-2011 (4) (a) (1)]			
30	Is each pH adjustment documented? [15A NCAC 2H .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-2011 (4) (b) (2)]			O-R (Oxidizing/Reducing agents) testing is not in the method. Adding <b>Potassium Bi-iodate</b> (not listed in method with chemicals used in test) to neutralize reducing agents is old practice. <b>Is not in method – must not be done.</b> 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.
32	How are the samples checked for chlorine? [SM 5210 B-2011 (4) (b) (2)] <b>ANSWER:</b>			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.
33	If chlorine is present, is it properly neutralized with sodium sulfite? [SM 5210 B-2011 (4) (b) (2)]			<b>See <a href="#">Neutralizing TRC in BOD Samples document</a>.</b>
34	Is this documented? This is considered pertinent information. [15A NCAC 2H .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-2011 (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 <sub>2</sub> O <sub>2</sub> ) present in any samples? [SM 5210 B-2011 (4) (b) (5)]			
37	If so, how is hydrogen peroxide treated? [SM 5210 B-2011 (4) (b) (5)]  <b>ANSWER:</b>			H <sub>2</sub> O <sub>2</sub> can cause supersaturated DOs. May see higher DO after 5 days than initial DO. <b>Treatment</b> – Mix samples vigorously in open container until H <sub>2</sub> O <sub>2</sub> dissipates. Check adequacy of H <sub>2</sub> O <sub>2</sub> 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.
	<b>PROCEDURE - Seeding</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
38	Are samples seeded if required? [SM 5210 B-2011 (4) (d)]			Samples needing seeding: <b>pH</b> outside acceptable range, <b>disinfected</b> (UV, chlorine, chlorinated/dechlorinated etc.), <b>industrial, high-temperature, wastes stored more than 6 hours after collection.</b> 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-2011 (4) (d)]  <b>ANSWER:</b>			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]  <b>ANSWER:</b>			<b>See <a href="#">Preparation of Synthetic Seed Material</a> document.</b>
41	If commercial seed is not used, how is it prepared? SM 5210 B-2011 (4) (d)]  <b>ANSWER:</b>			<b>Suitable sources:</b> 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-2011 (5) (d)]			
43	How many seed controls are analyzed? [SM 5210 B-2011 (6) (d)]  <b>ANSWER:</b>			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-2011 (7) (a) (1)]			<b>See <a href="#">Calculations and Reporting</a> document.</b>
45	Is sufficient seed material used in each seed control to yield a seed correction of 0.6 - 1.0 mg/L? [SM 5210 B-2011. (5) (d).]			The DO uptake attributable to the seed generally should be between 0.6 and 1.0 mg/L but the amount of seed added should be adjusted from this range to that required to provide GGA check results of 198 ± 30.5 mg/L. .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

46	If the calculated seed correction is between 1.0-1.4 on a regular basis, does the laboratory have data to show that this is required in order to obtain acceptable GGA values? [NC WW/GW LC Policy]			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).
47	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-2011 (7) (a) (1)]			
48	Are the results of all acceptable seed control analyses averaged? [SM 5210 B-2011 (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 ( $\pm 30\%$ ) 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.
	<b>PROCEDURE- CBOD<sub>5</sub></b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
49	If CBOD <sub>5</sub> is required, is a nitrification inhibitor added to all samples, seed controls and GGAs? [SM 5210 B-2011 (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-2001 (6) (c).
50	If BOD/CBOD samples are analyzed together, are separate seed controls and GGAs set up? [SM 5210 B-2011 (5) (e) and (6) (d)]			
51	What nitrification inhibitor is used for CBOD <sub>5</sub> samples? [SM 5210 B-2011 (5) (e)] <b>ANSWER:</b>			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).
52	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-2011 (5) (e) (1)]			
53	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-2011 (5) (e) (2)]			
54	Is the ATU prepared fresh every two weeks? [SM 5210 B-2011 (3) (g) (2)]			
55	What acceptance criterion is used for the CBOD GGA? [SM 5210 B-2011 (5) (e) or NC WW/GW LC Policy]			Method says it must be $198 \pm 30.5$ mg/L. Guidance from EPA Region IV says that it may be $164 \pm 30.7$ ). The lab must choose which acceptance criteria they will follow and not switch between them.
	$198 \pm 30.5$ (167.5 – 228.5)?			
	$164 \pm 30.7$ (133.3 – 194.7)?			
56	<b>All questions on checklist apply to CBOD – the above are just additional questions exclusive to CBOD</b>			
	<b>PROCEDURE- Sample Analysis</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
57	Is the initial DO of the blank at between 7.5 and 9.0 mg/l? [SM 5210 B-2011 (5) (a)]			Initial DO of blank must be at least 7.5 mg/L
58	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (B)] <b>ANSWER:</b>			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.

59	Is initial DO of all bottles other than blank between 7 and 9 mg/L? [SM 5210 B-2011 (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.
60	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (B)] <b>ANSWER:</b>			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.
61	Do documented times demonstrate that initial DOs are measured within 30 minutes of preparing dilutions? [SM 5210 B-2011 (5) (g)]			
62	Is the sample stirred during DO measurement or mixed immediately prior to measurement? [SM 5210 B-2011 (5) (f)]			The probe should be rinsed between bottles to prevent contamination. Older methods referenced this as a requirement (SM 20 <sup>th</sup> 5210 B (4)(f) Not mentioned in 5210 B-2011. May also read each sample's bottles from weaker to stronger without rinsing between each bottle. May cite as recommendation
63	Are at least three dilutions set for samples? [SM 5210 B-2011 (5) (c)]			Make at least three dilutions estimated to meet the 2-1 rule. Five dilutions are recommended for unfamiliar samples.
64	How are sample volumes measured? [NC WW/GW LC policy] <b>ANSWER:</b>			<b>NC WW/GW LC policy:</b> When measuring BOD/CBOD sample volumes: Sample volumes greater than or equal to 20 mls may be measured using a graduated cylinder. Sample volumes less than 20 mls must be measured using a <b>wide-tip pipet</b> . For dilutions greater than 1:100 make a primary dilution before making final dilution. This would be any measurements of less than <b>3 mls</b> sample volume if diluting directly into BOD bottles.
65	For dilutions with >67% sample (> 201 mL), are extra nutrients added? [SM 5210 B-2011 (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.
66	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-2011 (5) (g)]			
67	Are the samples incubated for five days ± 6 hours? [SM 5210 B-2011 (5) (h) and (i)]			
68	Are samples incubated in the dark at 20 ± 1 °C? [SM 5210 B-2011 (5) (h)]			
69	Are bottles completely filled and stoppered in such a manner that leaves no bubbles in the bottle? [SM 5210 B-2011 (5) (f)]			
70	Are water seals maintained on the bottles during incubation? [5210 B-2011 (5) (f)]			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.
71	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-2011 (7) (b)]			
72	Are seed corrections properly subtracted from the seeded samples? [SM 5210 B-2011 (7) (a) (1)]			See <a href="#">Calculations and Reporting</a> document.
73	Is the final BOD of the samples properly calculated? [SM 5210 B-2011 (7) (a) (1)]			See <a href="#">Calculations and Reporting</a> document.
	<b>QUALITY ASSURANCE</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>

74	Is the date/time samples are placed into the incubator documented? [15A NCAC 2H .0805 (a) (7) (F)]		<a href="#">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.</a>
75	Is the date/time samples are taken out of the incubator documented? [15A NCAC 2H .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.
76	Are incubator temperatures documented? [15A NCAC 2H .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 $20 \pm 1$ °C
77	Is a dilution water blank analyzed? [SM 5210 B-2011 (6) (c)]		
78	Is the dilution water blank depletion $\leq 0.20$ mg/L? [SM 5210 B-2011 (6) (c)]		<p>Preferably <math>&lt; 0.10</math> mg/L. Blank readings must 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. If the lab would like something in writing to help justify getting a new meter, we can point to this.</p> <p>Multiple dilution water blanks in the same batch using the same dilution water are to be treated as replicates and averaged. The <u>average</u> of the dilution water blanks in a <b>batch</b> must not be <math>&gt;0.20</math> mg/L. (<b>See attached SM Memo dated August 1, 2014</b>).</p> <p>We define a <b>batch</b> as an individual storage container of dilution water used in a single day. That is because both the quality of the water and the cleanliness of the storage container affect the value of the blank. Therefore, a blank is required for each storage container of water.</p> <p><b>Sample results associated with blanks reading <math>&gt;0.20</math> mg/L must be qualified.</b> 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.</p> <ul style="list-style-type: none"> <li>• If more than one blank is analyzed for a single container of water, the average of the blanks must be <math>\leq 0.20</math> mg/L.</li> <li>• If more than one container of water is used, a blank must be analyzed for each container of water. That blank (or the average of multiple blanks) must be <math>\leq 0.20</math> mg/L.</li> </ul> <p><b>See <a href="#">Flagging QC Failures</a> document.</b></p>
79	If not, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (B)]  <b>Answer:</b>		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.
80	Is a seeded blank analyzed? (Recommendation)		Not in methods – recommended – should approximate the final SCF
81	What is the average typical difference between the seeded blank and the calculated seed correction factor? <b>Answer:</b>		$\approx \leq 0.2$ 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.
82	Are three bottles of glucose-glutamic acid standard (GGA) analyzed with each set of samples? [SM 5210 B-2011 (6) (b)]		
83	What is the source of the GGA standard? [SM 5210 B-2011 (3) (h) and (6) (b)] <b>Answer:</b>		May make or buy prepared. If making, dry reagents at 103 °C for 1 hr.
84	How frequently is the GGA standard made? [SM 5210 B-2011 (3) (h)] <b>Answer:</b>		May be held if maintained in sterile conditions and stored at $\leq 4$ °C ( $\leq 6$ °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 mL into sample bottle then discard any excess. Should not try to hold longer than approximately 1 month. Look at GGA recoveries to see if it is degrading.
85	Is the GGA standard analyzed at a 2% dilution? [SM 5210 B-2011 (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. 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 adjust dosage before setting up, that is must dilute 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.
86	Is the GGA standard seeded? [SM 5210 B-2011 (8)]		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.
87	Is the average of the three GGA bottles $198 \pm 30.5$ mg/L? [SM 5210 B-2011 (6) (b)]		
88	Is the average of the three GGA bottles $164 \pm 30.7$ mg/L for CBOD <sub>5</sub> ? [SM 5210 B-2011 (5) (e)-2001 or NC WW/GW LC Policy]		Method says it must be $198 \pm 30.5$ mg/L. Guidance from EPA Region IV says that it may be $164 \pm 30.7$ . The lab must choose which acceptance criteria they will follow and not switch between them.
89	If the GGA is not acceptable, what corrective action is taken? [15A NCAC 2H .0805 (a) (7) (B)] <b>Answer:</b>		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.
90	Is a duplicate analyzed daily or with each batch of 20 or fewer samples? [SM 5020 B-2010 (2) (f)]		Only have to duplicate one dilution of the three set up to meet minimum requirement. Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples. We interpret matrix type to be aqueous and non-aqueous.
91	Do duplicates check within 30% of each other? [SM 5210 B-2011 (7) (b)]		Duplicates having concentrations greater than the lower reporting limit of 2.0 mg/L must check within 30%. <b>See <a href="#">NC WW/GW LC Policy for Flagging QC Failures</a> document.</b>
92	Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [15A NCAC 2H .0805 (a) (7) (B)]		<b>See <a href="#">NC WW/GW LC Policy for Flagging QC Failures</a> document.</b>  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.
93	What is the reporting limit (PQL)? [NC WW/GW LC Policy]  <b>Answer:</b>		NC WW/GW LC policy is 2 mg/L based on SM 5210 B. (6) (a)-2001. Only bottles, including seed controls, giving a minimum 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 allowed to elevate the PQL and report as less than the value that would have been obtained if the dilution had depleted the required 2.0 mg/L. This is due to the requirement that you flag the data if it does not deplete the 2.0 mg/L versus elevating the reporting limit to avoid flagging. <b>See <a href="#">Calculations and Reporting</a> document. See example #3 regarding elevating reporting limit.</b>

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<sub>2</sub>SO<sub>3</sub> in 100 mL distilled water. Prepare fresh daily.
2. 2% H<sub>2</sub>SO<sub>4</sub> - Add 2 mL concentrated H<sub>2</sub>SO<sub>4</sub> 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 SM 4500 O C (2) (d)-2001.

### Procedure:

To 100 mL of sample, add approximately 1 mL of 2% H<sub>2</sub>SO<sub>4</sub>, 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

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

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

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

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

**15 mL seed control yields:** initial DO 8.1mg/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 8.1mg/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.

## 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  $\geq 2.0$  mg/L DO depletion and  $\geq 1.0$  mg/L residual DO (“2 and 1 rule”), calculate BOD as follows according to SM 5210 B (7) (a)-2001:

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

$D_1$  = 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

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 <sub>5</sub> 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	<b>12.4</b>
8.0	1.5	6.5	0.8	5.7	150	2	<b>11.4</b>
8.0	0.13	7.87	0.8	7.07	300	1	>7.07

\* Final DO must be  $\geq 1.0$  mg/L in order to meet the “2 and 1 rule”. When reviewing data, all values in this column should be  $\geq 1.0$  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  $\geq 2.0$  mg/L in order to meet the “2 and 1 rule”. When reviewing data, all values in this column should be  $\geq 2.0$  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  $\geq 2.0$  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”:****Example Calculation #2 – 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 5210 B. (6) (a)-2001]

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 <sub>5</sub> 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	<b>1.0</b>

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

**Example Calculation #3 – 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 <sub>5</sub> 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  $\geq 2.0$  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.

**Example Calculation #4 – 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 <sub>5</sub> mg/L
8.00	0.35	7.65	0.80	6.85	6	50	<b>&gt;342.5</b>
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).

**NC WW/GW LC Policy for Flagging BOD Quality Control Failures** (NC WW/GW LC policy 08/26/2014)

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) or in the comment section for an eDMR. 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 SM 5210 B (7) (b)-2001 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. Multiple dilution water blanks in the same batch using the same dilution water are to be treated as replicates and averaged. The average of the dilution water blanks in a batch must not be more than 0.20 mg/L.
3. The average of the three Glucose - Glutamic Acid (GGA) check standards falls outside the acceptance limits [i.e., 198 mg/L  $\pm$  30.5 mg/L (167.5 – 228.5 mg/L) or 164  $\pm$  30.7 mg/L (133.3 – 194.7 mg/L) for CBOD].
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.



**STANDARD METHODS**  
FOR THE  
**EXAMINATION OF WATER AND WASTEWATER**  
JOINT EDITORIAL BOARD  
**MEMORANDUM**

**To:** *Standard Methods Users*  
Biochemical Oxygen Demand (BOD)

*Andrew Eaton*  
**From:** Andrew Eaton  
Joint Editorial Board

**Re:** BOD Multiple Dilution Water Blanks      **Date:** August 1, 2014

This memo is in response to questions about how to determine acceptance criteria when multiple Dilution Water Blanks are performed in the *Standard Methods* 5210B – 2011 method.

The method states in section 5210B.6.c: “With each batch of samples incubate one or more bottles of dilution water that contains nutrient, mineral and buffer solutions but no seed or nitrification inhibitor. This dilution water blank serves as a check on quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final DO as in 5210B.5g and i. The DO uptake in 5 d must not be more than 0.20 mg/L and preferably not more than 0.10 mg/L, before making seed corrections. If the dilution water blank exceeds 0.20 mg/L, discard all data for tests using this dilution water or clearly identify such samples in data records.”

The method does not specifically address how to handle multiple dilution water blanks.

After JEB discussion with the Part Coordinator and Joint Task Group chair for Section 5210, it has been determined that multiple Dilution Water Blanks in the same batch using the same dilution water are to be treated as replicates and averaged. The average of the dilution water blanks in a batch must not be more than 0.20 mg/L. We will include this information in the next published version of the method.

cc: Robin Parnell (Part Coordinator – Part 5000)

### 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  $\pm 0.5$  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:

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