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: **Fecal (Thermotolerant) Coliform Sludge MPN (Biosolids)**
Method: **SM 9221 E-2014**

Equipment:

	Evaporating Dishes 100 mL capacity, 90 mm diameter made of Porcelain, Platinum or high silica glass			Analytical balance, capable of weighing 0.1 mg (0.0001g)		Desiccator, provided with desiccant containing a color indicator of moisture concentration or an instrumental indicator
	Drying oven, for operation at 103 to 105 ºC			Graduated Cylinder		Alcohol
	Pan balance capable of weighing 0.1 g			Sterilizer oven, 170ºC		Autoclave, capable of maintaining 121ºC for 15 minutes
	Incubator, 35 ± 0.5ºC			Water bath, 44.5 ± 0.2ºC		Thermometer, 0.5ºC increments
	Thermometer, 0.1ºC increments			Sterile wood applicator sticks		Refrigerator
	Inoculation loop			Sterile blender		No. 7 cork borer (not required)

Reagents:

	EC medium		A-1 medium
	Lauryl Tryptose broth (single strength)		Buffered dilution water
	Lauryl Tryptose broth (double strength)		Bromocresol purple (not required)
	Sterile reagent water		

Note: Lauryl Sulfate may be substituted for Lauryl Tryptose.

PLEASE COMPLETE CHECKLIST IN INDELIBLE INK
Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

	GENERAL	LAB	SOP	EXPLANATION
1	Is the SOP reviewed at least every 2 years? What is the most recent review/revision date of the SOP? [15A NCAC 2H .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. **SM 9020 A-2015 states: QC requirements in section 9020 are not mandatory.** Each laboratory must develop its own QC suitable for its needs and, in some cases, as required by regulatory agencies, standard setting organizations, and laboratory certification or accreditation programs. The program must be practical and require only a reasonable amount of time or it will be bypassed. Once a QA program is established, about **15%** of overall laboratory time should be spent on different aspects of the program. When properly administered, a balanced, conscientiously applied quality system will optimize data quality, identify problems early, and increase satisfaction with the analytical results without adversely affecting laboratory productivity. SM 9020 A-2015 (4) states: The QC guidelines discussed in 9020 B and 9020 C are recommended as useful source material of elements that need to be addressed in developing policies for a QA program and QC activities.

				Based upon this language, in conjunction with method specified requirements, the NC WW/GW LC program has established minimum requirements for maintaining certification from our program. These are addressed in this checklist along with recommendations to be considered as the laboratory's QC program evolves over time.
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	**LAB**	**SOP**	**EXPLANATION**
4	Are samples collected in sterile bottles? [40 CFR 136.3 Table II]			
5	Are samples iced to < 10 ºC during shipment? [40 CFR 136.3 Table II]			
6	Are samples stored at < 10 ºC prior to analysis? [40 CFR 136.3 Table II]			
7	Are samples analyzed as soon as possible after collection with the start of incubation no more than 8 hours after collection? [40 CFR 136.3 Table II]			Footnote 23 extends the holding time to 24 hours for Class A composted, Class B aerobically digested, and Class B anaerobically digested samples **only when analyzed according to EPA Methods 1680 (LTB–EC) and 1681 (A–1).**
	PROCEDURE- Sample Collection	**LAB**	**SOP**	**EXPLANATION**
8	Are at least 2 samples collected (≥7 recommended) from each source for Class A sludge? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Section 10.7 and Appendix F. (1) (1.2)] **How many samples are collected?** **Over what time frame (2-week period recommended) are samples collected?**			1.2 for Class A States: Part 503 requires that, to qualify as a Class A sludge, treated sewage sludge must be monitored for fecal coliform (or Salmonella sp.) and have a density of less than 1,000 MPN fecal coliform per gram of total solids (dry weight basis). **The regulation does not specify total number of samples. However, it is suggested that a sampling event extend over two weeks and that at least seven samples be collected and analyzed**. The total solids content for each sample must be determined in accordance with procedure 2540 G. of SM. 10.7, Class A states: The regulation does not specify the number of samples that have to be taken during a monitoring event. One sample is not enough to properly represent the sewage sludge.
9	Are at least 7 samples collected from each source for Class B sludge? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Chapter 9, Section 9.5] [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Section 10.7 and Appendix F. (1) (1.1)] **How many samples are collected?** **Over what time frame (2-week period recommended) are samples collected?**			1.1 for Class B States: To demonstrate that a given domestic sludge sample meets Class B Pathogen requirements under alternative 1, the density of fecal coliform from at least seven samples of treated sewage sludge must be determined and the geometric mean of the fecal coliform density must not exceed 2 million Colony Forming Units (CFU) or Most Probable Number (MPN) per gram of total solids (dry weight basis). Chapter 9, Section 9.5 states: To meet Class B Alternative 1 requirements, **seven samples must be taken** and the geometric mean of results must meet the 2.0×10^6 MPN fecal coliform per dry gram limit (see Chapter 5). **It is recommended that the samples be taken over a two-week period in order to adequately represent variability in the sewage sludge.**
	PROCEDURE- Dry Weight Determination	**L**	**S**	**EXPLANATION**

		A B	O P	
10	Is an aliquot of each of the samples dried and the solids content determined in accordance with Standard Method 2540 G-2015? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1) (1.1) and (1) (1.2)]			1.1 for Class B States: To demonstrate that a given domestic sludge sample meets Class B Pathogen requirements under alternative 1, the density of fecal coliform from at least seven samples of treated sewage sludge must be determined and the geometric mean of the fecal coliform density must not exceed 2 million Colony Forming Units (CFU) or Most Probable Number (MPN) per gram of total solids (dry weight basis). The solids content of treated domestic sludge can be highly variable. Therefore, an aliquot of each sample must be dried and the solids content determined in accordance procedure 2540 G. of the 18th edition of Standard Methods for the Examination of Water and Wastewater (SM). Note: EPA/625/R-92/013 has not been updated since 2003. Laboratories are to use the version of SM 2540 G that coincides with other approved residue methods. 1.2 for Class A States: Part 503 requires that, to qualify as a Class A sludge, treated sewage sludge must be monitored for fecal coliform (or Salmonella sp. and have a density of less than 1,000 MPN fecal coliform per gram of total solids (dry weight basis). The regulation does not specify total number of samples. However, it is suggested that a sampling event extend over two weeks and that at least seven samples be collected and analyzed. The total solids content for each sample must be determined in accordance with procedure 2540 G. of SM.
11	Are sterile evaporating dishes dried at 104 ±1ºC for one hour and cooled in a desiccator and weighed? [SM 2540 G-2015 (3) (a) (1)]			Heat dish at 103 - 105 ºC for ≥1 hr in an oven. Cool in desiccator, weigh, and store in desiccator or 103 - 105 ºC oven until ready for use.
12	Is sample manually processed? [SM 2540 G-2015 (3) (a) (2) (b)]			Manually process samples as quickly as possible to prevent moisture loss. Processing via mechanical grinding is not recommended because moisture levels could drop during processing.
13	Are 25-50 grams of mixed, cored or coarsely pulverized sample placed in a prepared evaporating dish and weighed? [SM 2540 G-2015 (3) (a) (2) (b)]			If the sample consists of discrete pieces of solid material (dewatered sludge, for example), take cores from each piece with a No. 7 cork borer or pulverize the entire sample coarsely on a clean surface by hand, using rubber gloves. Place 25 to 50 g in a prepared evaporating dish and weigh.
14	Is the sample dried in a 103 - 105 ºC oven for ≥1 hr? [SM 2540 G-2015 (3) (a) (2) (b)]			Place in an oven at 103 to 105°C for ≥1 hr.
15	Is the sample cooled to balance temperature in a desiccator and weighed? [SM 2540 G-2015 (3) (a) (2) (b)]			Cool to balance temperature in a desiccator and weigh.
16	Is the cycle repeated (sample dried again for ≥1 hour, cooled in a desiccator and weighed) until weight change is <50mg? [SM 2540 G-2015 (3) (a) (2) (b)]			
17	Are all times that samples are placed into and removed from the oven 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
18	How are the % total solids calculated? [SM 2540 G-2015 (4)] **ANSWER:**			$\frac{(A - B) \times 100}{C - B}$ A = weight of dried residue + dish, mg B = weight of dish, mg C = weight of wet sample + dish, mg
	LTB Medium Preparation	**L A B**	**S O P**	**EXPLANATION**

19	Is the LTB medium purchased ready-to-use or prepared in the lab? If prepared in the lab, skip to question 22.			Although SM 9221 B-2014 (3) provides instructions for preparing medium from individual components, a commercially prepared mix of the dehydrated medium must be used if prepared in the lab since it is readily available. Alternatively, the medium may be purchased ready-to-use and already dispensed into tubes with inverted vials.
20	If purchased ready-to-use media is used with a manufacturer's expiration date that exceeds the holding time stated in SM 9020 B-2015, Table 9020: V, is the manufacturer's statement of quality to that extended time on file? [SM 9020 B-2015 (5) (j) (4)]			SM 9020 B-2015 (5) (j) (4) states: If prepared ready-to-use commercial medium has an expiration date greater than that noted in Table 9020:V, have the manufacturer supply evidence of medium quality for that entire period . Verify usability weekly by testing recoveries with known densities of culture controls that will also meet QC check requirements.
21	If no manufacturer's data is available, is a culture positive with known densities analyzed weekly to demonstrate viability? [SM 9020 B-2015 (5) (j) (4)] If using EC medium, skip to question 33. If using A-1 medium, skip to question 46.			See explanation above.
22	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			SM 9020-2015 B (5) (j) (1) Page 9-16 states: Document preparation activities such as name of media, volume produced, format, final pH, date prepared, and name of preparer.
23	Is media prepared in clean containers that are at least twice the volume of the media being prepared? [SM 9020 B-2015 (5) (j) (1)]			
24	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
25	Is media stirred while heating? [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) (1) states: Stir media, particularly agars, while heating. Avoid scorching or boil-over by using a boiling water bath for small batches of media and by continually attending to larger volumes heated on a hot plate or gas burner. Preferably use hot plate stirrer combinations.
26	Is sufficient medium dispensed in fermentation tubes with an inverted vial (Durham tube) to cover the inverted vial at least one-half to two-thirds after sterilization? [SM 9221 B-2014 (3) (a)]			Before sterilization, dispense sufficient medium, in fermentation tubes with an inverted vial, to cover the inverted vial at least one-half to two-thirds after sterilization.
27	If a Durham tube is omitted, is 0.01 g/L of bromocresol purple added to the LTB? [SM 9221 B-2014 (3) (a)]			Alternatively, omit the inverted vial and add 0.01 g/L bromocresol purple to lauryl tryptose broth to determine acid production, an indicator of a positive result in this part of the coliform test.3
28	Is medium autoclaved at 121°C for 12 to 15 minutes in capped tubes? [SM 9221 B-2014 (3) (a)]			Close tubes with metal or heat-resistant plastic caps. Autoclave medium at 121°C for 12 to 15 min. SM 9020 B-2015 (5) (j) (2) states: Do not expose media containing carbohydrates to the elevated temperatures for more than 45 min. Exposure time is defined as the period from initial exposure to heat to removal from the autoclave. Overheating of media can result in nutrient degradation. Note: Cap tubes loosely and set autoclave exhaust to slow.
29	After sterilization, are inverted vials free of air bubbles? [SM 9221 B-2014 (3) (a)]			Ensure that inverted vials are free of air bubbles.
30	Is pH of the LTB medium adjusted if necessary and documented to be 6.8 ± 0.2 S.U.? [SM 9221 B-2014 (3) (a) and SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) states: Check and record pH of a portion of each media after sterilization. Adjustment of pH will seldom be necessary when commercially available media are used. If needed, make minor adjustments to the pH specified in the formulation with filter-sterilized 1*N* NaOH or 1*N* HCl solutions. If the pH difference is larger than 0.5 units, discard the batch and check preparation instructions and pH of reagent water to resolve the problem. If medium is known as requiring pH adjustment, adjust pH appropriately prior to sterilization and record final

				pH. SM 9221 B-2014 (3) (a) states: Medium pH should be 6.8 ± 0.2 after sterilization. Bottom line: It is required to check and document the pH of each batch of prepared media after sterilization. If the pH is not 6.8 ± 0.2 S.U. it must be adjusted to that range. Use 1*N* NaOH or 1*N* HCl that has been filtered and sterilized. If the pH is more than 0.5 S.U. outside of the specified pH, discard and determine why (e.g., incorrect preparation or abnormal pH of reagent water).
31	Is the LTB media used within the container-specific holding time? [SM 9020 B-2015 (5) (j) (1) Table 9020: V]			TABLE 9020:V. HOLDING TIMES FOR PREPARED MEDIA Medium — Holding Time Broth in screw-cap flasks* — 96 h Poured agar in plates with tight-fitting covers* — 2 weeks Agar or broth in loose-cap tubes* — 2 weeks Agar or broth in tightly closed screw-cap tubes† — 3 months Poured agar plates with loose-fitting covers in sealed plastic bags* — 2 weeks Large volume of agar in tightly closed screw-cap flask or bottle* — 3 months * Hold under refrigerated conditions 2–8°C. † Hold at <30°C.
32	Is the LTB media properly stored as described in Table 9020:V? [SM 9020 B-2015 (5) (j) (1) Table 9020: V] If using A-1 Medium, skip to question 46.			See Table in Explanation above
	EC Medium Preparation	**LAB**	**SOP**	**EXPLANATION**
33	Is the EC medium purchased ready-to-use or prepared in the lab? If purchased ready-to-use skip to question 43.			Although SM 9221 E-2014 (1) (a) provides instructions for preparing medium from individual components, a commercially prepared mix of the dehydrated medium must be used if prepared in the lab since it is readily available. Alternatively, the medium may be purchased ready-to-use and already dispensed into tubes with inverted vials.
34	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			SM 9020-2015 B (5) (j) (1) Page 9-15 states: Document preparation activities such as name of media, volume produced, format, final pH, date prepared, and name of preparer.
35	Is media prepared in clean containers that are at least twice the volume of the media being prepared? [SM 9020 B-2015 (5) (j) (1)]			
36	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
37	Is media stirred while heating? [SM 9020 B-2015 (5) (j) (1)]			Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. SM 9020 B-2015 (5) (j) (1) states: Stir media, particularly agars, while heating. Avoid scorching or boil-over by using a boiling water bath for small batches of media and by continually attending to larger volumes heated on a hot plate or gas burner. Preferably use hot plate stirrer combinations.
38	Is sufficient medium dispensed in fermentation tubes with an inverted vial (Durham tube) to cover the inverted vial at least one-half to two-thirds after sterilization? [SM 9221 E-2014 (1) (a)]			Before sterilization, dispense sufficient medium, in fermentation tubes with an inverted vial, to cover the inverted vial at least one-half to two-thirds after sterilization.
39	Is medium autoclaved at 121°C for 12 to 15 minutes in capped tubes? [SM 9221 E-2014 (1) (a)]			Close tubes with metal or heat-resistant plastic caps. Autoclave medium at 121°C for 12 to 15 min. SM 9020 B-2015 (5) (j) (2) states: Do not expose media containing carbohydrates to the elevated temperatures for more than 45 min. Exposure time is defined as the period from initial exposure to heat to removal from the autoclave. Overheating of media can result in nutrient degradation.

				Note: Cap tubes loosely and set autoclave exhaust to slow.
40	After sterilization, are inverted vials free of air bubbles? [SM 9221 E-2014 (1) (a)]			Ensure that inverted vials are free of air bubbles.
41	Is pH of the EC medium adjusted if necessary and documented to be 6.9 ± 0.2 S.U.? [SM 9221 E-2014 (1) (a)] and [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) states: Check and record pH of a portion of each media after sterilization. Adjustment of pH will seldom be necessary when commercially available media are used. If needed, make minor adjustments to the pH specified in the formulation with filter-sterilized 1*N* NaOH or 1*N* HCl solutions. If the pH difference is larger than 0.5 units, discard the batch and check preparation instructions and pH of reagent water to resolve the problem. If medium is known as requiring pH adjustment, adjust pH appropriately prior to sterilization and record final pH. SM 9221 E-2014 (1) (a) states: Medium pH should be 6.9 ± 0.2 S.U. after sterilization. It is required to check and document the pH of each batch of prepared media after sterilization. If the pH is not 6.9 ± 0.2 S.U. it must be adjusted to that range. Use 1*N* NaOH or 1*N* HCl that has been filtered and sterilized. If the pH is more than 0.5 S.U. outside of the specified pH, discard and determine why (e.g., incorrect preparation or abnormal pH of reagent water).
42	Is the EC media used within the container-specific holding time? [SM 9020 B-2015 (5) (j) (1) Table 9020: V] Skip to question 58.			TABLE 9020:V. HOLDING TIMES FOR PREPARED MEDIA Medium — Holding Time Broth in screw-cap flasks* — 96 h Poured agar in plates with tight-fitting covers* — 2 weeks Agar or broth in loose-cap tubes* — 2 weeks Agar or broth in tightly closed screw-cap tubes† — 3 months Poured agar plates with loose-fitting covers in sealed plastic bags* — 2 weeks Large volume of agar in tightly closed screw-cap flask or bottle* — 3 months * Hold under refrigerated conditions 2–8°C. † Hold at <30°C.
43	If purchased ready-to-use media is used with a manufacturer's expiration date that exceeds the holding time stated in SM 9020 B-2015, Table 9020: V, is the manufacturer's statement of quality to that extended time on file? [SM 9020 B-2015 (5) (j) (4)]			SM 9020 B-2015 (5) (j) (4) states: If prepared ready-to-use commercial medium has an expiration date greater than that noted in Table 9020:V, have the manufacturer supply evidence of medium quality for that entire period . Verify usability weekly by testing recoveries with known densities of culture controls that will also meet QC check requirements.
44	If no manufacturer's data is available, is a culture positive with known densities analyzed weekly to demonstrate viability? [SM 9020 B-2015 (5) (j) (4)]			See explanation above.
45	Is the EC media properly stored as described in Table 9020:V? [SM 9020 B-2015 (5) (j) (1) Table 9020: V] Skip to question 58.			See table in question # 43 explanation above.
	A-1 Medium Preparation	**LAB**	**SOP**	**EXPLANATION**
46	Is the A-1 medium purchased ready-to-use or prepared in the lab? If prepared in the lab, skip to question 49.			Although SM 9221 E-2014 (2) (a) provides instructions for preparing medium from individual components, a commercially prepared mix of the dehydrated medium must be used if prepared in the lab since it is readily available. Alternatively, the medium may be purchased ready-to-use and already dispensed into tubes with inverted vials.
47	If purchased ready-to-use media is used with a manufacturer's expiration date that exceeds the holding time stated in SM 9020 B-2015, Table 9020: V, is the manufacturer's statement of quality to that extended time on file? [SM 9020 B-2015 (5) (j) (4)]			SM 9020 B-2015 (5) (j) (4) states: If prepared ready-to-use commercial medium has an expiration date greater than that noted in Table 9020:V, have the manufacturer supply evidence of medium quality for that entire period . Verify usability weekly by testing

				recoveries with known densities of culture controls that will also meet QC check requirements.
48	If no manufacturer's data is available, is a culture positive with known densities analyzed weekly to demonstrate viability? [SM 9020 B-2015 (5) (j) (4)] Skip to question 58.			See explanation above.
49	Is media prepared in clean containers that are at least twice the volume of the media being prepared? [SM 9020 B-2015 (5) (j) (1)]			
50	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
51	Is media stirred while heating? [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) (1) states: Stir media, particularly agars, while heating. Avoid scorching or boil-over by using a boiling water bath for small batches of media and by continually attending to larger volumes heated on a hot plate or gas burner. Preferably use hot plate stirrer combinations.
52	Are both a single strength and double strength medium prepared? [SM 9221 E-2014 (2) (a)]			For 10 ml samples, prepare double-strength medium so the final concentration of ingredients after sample addition is correct.
53	Is pH of the A-1 medium adjusted if necessary and documented to be 6.9 ± 0.1 S.U.? [SM 9221 E-2014 (2) (a)] and [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) states: Check and record pH of a portion of each media after sterilization. Adjustment of pH will seldom be necessary when commercially available media are used. If needed, make minor adjustments to the pH specified in the formulation with filter-sterilized 1*N* NaOH or 1*N* HCl solutions. If the pH difference is larger than 0.5 units, discard the batch and check preparation instructions and pH of reagent water to resolve the problem. If medium is known as requiring pH adjustment, adjust pH appropriately prior to sterilization and record final pH. SM 9221 E-2015 (2) (a) states: …adjust to pH 6.9 ± 0.1. It is required to check and document the pH of each batch of prepared media after sterilization. If the pH is not 6.9 ± 0.1 S.U. it must be adjusted to that range. Use 1*N* NaOH or 1*N* HCl that has been filtered and sterilized. If the pH is more than 0.5 S.U. outside of the specified pH, discard and determine why (e.g., incorrect preparation or abnormal pH of reagent water).
54	Are fermentation tubes, containing an inverted vial, filled with sufficient medium to cover the inverted vial at least one-half to two-thirds after sterilization? [SM 9221 E-2014 (2) (a)]			Before sterilization dispense, in fermentation tubes with an inverted vial, sufficient medium to cover the inverted vial at least one-half to two-thirds after sterilization.
55	Are the tubes closed with metal or heat-resistant plastic caps and sterilized by autoclaving at 121°C for 10 min? [SM 9221 E-2014 (2) (a)]			Close with metal or heat-resistant plastic caps. Sterilize by autoclaving at 121°C for 10 min.
56	Are the inverted vials verified to be free of air bubbles? [SM 9221 E-2014 (2) (a)]			Ensure that inverted vials are free of air bubbles.
57	Are sterilized A-1 tubes stored at room temperature in the dark and used within 7 days? [SM 9221 E-2014 (2) (a)]			Store in the dark at room temperature for not longer than 7 days. Ignore formation of precipitate during storage.
	Sterile Buffered Dilution Water Preparation	**LAB**	**SOP**	**EXPLANATION**
58	How is the sterile rinse/dilution water prepared? [SM 9050 C-2015 (1) (a)] **ANSWER:**			Add 1.25 mL stock Phosphate buffer solution and 5.0 ml magnesium chloride stock solution to 1-L reagent grade water. 100 ml volumes or less autoclave for 15 minutes. Rinse water volumes >100 ml adjust autoclave time for volume – see table 9020: IV, SM 9020 B-2015. Final pH should be 7.2 ± 0.1 S.U. Recommended but not required to check pH. Recommend checking if performing

				troubleshooting due to suspected issues. Note that pH values will change with time. Store under refrigerated conditions after opening and discard if turbidity develops. Use within 6 months. If dilutions are prepared – do not suspend a sample in any dilution water for more than 30 minutes at room temperature because injury, death, or multiplication may occur.
59	Are the Phosphate buffer and Magnesium Chloride stock solutions sterilized after preparation and stored in the refrigerator? [SM 9050 C-2015 (1) (a) (1) and (2)]			Stock Phosphate buffer solution; Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 ml reagent grade water, adjust to pH 7.2 ± 0.5 with 1N NaOH and dilute to 1 L with reagent grade water. Sterilize by filtration or autoclave. Store stock solution under refrigerated conditions and discard if turbidity develops. Magnesium chloride stock solution: Add magnesium chloride (38 g/L $MgCl_2$ or 81.1 g $MgCl_2$ - $6H_2O$) to 1 l reagent grade water. Sterilize and store stock solution under refrigerated conditions, discarding if solution becomes turbid.
60	Is the stock phosphate buffer documented to be pH 7.2 ± 0.5 S.U.? This is considered pertinent information. [SM 9050 C-2015 (1) (a) (1)] and [15A NCAC 2H .0805 (a) (7) and (a) (7) (E)]			If prepared, document in the preparation instructions or if purchased, retain manufacturer's documentation stating it is the proper pH. Rule: All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
	PROCEDURE – Sample Preparation	**LAB**	**SOP**	**EXPLANATION**
It is recommended that laboratories analyzing unfamiliar samples set 5 dilutions instead of 4.				
61	When analyzing a solid sample, is 30.0 grams of well mixed sample, containing all materials which will be included in the sludge, used for each sample? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), solid samples (1)]			In a sterile dish weigh out 30.0 grams of well mixed sample. Whenever possible, the sample tested should contain all materials which will be included in the sludge. For example, if wood chips are part of a sludge compost, some mixing or grinding means may be needed to achieve homogeneity before testing. One exception would be large pieces of wood which are not easily ground and may be discarded before blending. Analyzing less than 30.0 g of sample is not permitted.
62	When analyzing a liquid sample, is 30.0 mL of well mixed sample, containing all materials which will be included in the sludge, used for each sample? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (1)]			In a sterile graduated cylinder, measure out 30.0 mL of well mixed sample. Whenever possible, the sample tested should contain all materials which will be included in the sludge. Analyzing less than 30.0 mL of sample is not permitted.
63	Is the sample transferred to a sterile blender and 270 mL of sterile buffered dilution water used to rinse any remaining sample in the weighing dish or graduated cylinder into the blender? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), solid samples (1) and liquid sample (1)]			Use 270 mL of sterile buffered dilution water to rinse any remaining sample into the blender.
64	Is the sample homogenized in the blender at high speed for two minutes? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), solid samples (1) and liquid sample (1)]			Cover and blend on high speed for two minutes. **1 ml of this sample contains 0.10 g of the original sample.**
65	Is a sterile pipette used to transfer 11.0 mL of the blender contents to a screw cap bottle containing 99 mL of sterile buffered dilution water? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003)			Use a sterile pipette to transfer 11.0 mL of the blender contents to a screw cap bottle containing 99 mL of sterile buffered dilution water. **This becomes dilution "A."**

	Appendix F. (1.1), solid samples (2) and liquid sample (2)]			
66	Is this sample (known as dilution "A") shaken vigorously a minimum of 25 times? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), solid samples (2) and liquid sample (2)]			Shake vigorously a minimum of 25 times. One milliliter of this sample contains 0.010 g of the original sample. 1.0 mL of this mixture is 0.010 mL of the original sample.
67	Is a sterile pipette used to transfer 1.0 mL of dilution "A" to a second screw cap bottle containing 99 mL of sterile buffered dilution water to create dilution "B", and mixed as before? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (3)]			Use a sterile pipette to transfer 1.0 mL of dilution "A" to a second screw cap bottle containing 99 mL of sterile buffered dilution water and mix as before. **This becomes dilution "B."** 1.0 m L of this mixture is 0.00010 mL of the original sample.
68	Is a sterile pipette used to transfer 1.0 mL of dilution "B" to a second screw cap bottle containing 99 mL of sterile buffered dilution water to create dilution "C", and mixed as before? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (4)]			Use a sterile pipette to transfer 1.0 mL of dilution "B" to a second screw cap bottle containing 99 mL of sterile buffered dilution water and mix as before. **This is dilution "C."**
	PROCEDURE – Sample Analysis w/ LTB and EC Medium	**LAB**	**SOP**	**EXPLANATION**
69	Are four series of 5 LTB tubes used for the analysis? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)] If using A-1 media, skip to question 79.			Four series of 5 tubes will be used for the analysis.
70	Is the concentration of the LTB used in each series based on the amount of inoculum added? [SM 9221 B-2014 (3) (a)]			Prepare in accordance with Table 9221:I; making lauryl tryptose broth concentrated enough that adding 100, 20, or 10mL portions of sample to the medium will not reduce ingredient concentrations below those of the standard medium.
71	For Class A samples: Is the first series of 5 tubes inoculated with 10 mL of the blender contents? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.2), liquid samples (1) and solid samples (1)]			Prepare the sample as described for "Class B Alternative 1, Liquid Samples *(or Solid Samples),"* except inoculate each of the first series of 5 tubes with 10.0 mL of the blender contents (the concentration of the enrichment broth must be adjusted to compensate for the volume of added sample). [Liquid Samples] This is equivalent to adding 1.0 mL of sludge to the first series of tubes. [Solid Samples] This is equivalent to adding 1.0 g of sludge (wet weight) to the first series of tubes.
72	For Class A samples: Are the remaining series of tubes inoculated using tenfold serial dilutions? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.2), liquid samples (1) and solid samples (1)]			Inoculate at least four series of five tubes using tenfold serial dilutions.
73	For Class B samples: Is the first series of 5 tubes each inoculated with 10.0 ml of dilution "B"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			Inoculate the first series of 5 tubes each with 10.0 mL of dilution "B." This is a 0.0010 m L of the original sample.
74	For Class B samples: Is the second series of 5 tubes each inoculated with 1.0 ml of dilution "B"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			The second series of tubes should be inoculated with 1.0 mL of dilution "B" (0.00010).
75	For Class B samples: Is the third series of 5 tubes each inoculated with 10.0 ml of dilution "C"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013,			The third series of tubes should receive 10.0 mL of "C" (0.000010).

	(July 2003) Appendix F. (1.1), liquid samples (5)]			
76	For Class B samples: Is the fourth series of 5 tubes each inoculated with 1.0 ml of dilution "C"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			Inoculate a fourth series of 5 tubes each with 1.0 mL of dilution "C" (0.0000010). Continue the procedure as described in SM.
77	Are all samples incubated at 35 ± 0.5ºC for 24 ± 2 hr? [SM 9221 B-2014 (3) (b) (2)]			
78	After 24 ± 2 hr, are tubes gently swirled and examined for growth, gas and/or acidity (shades of yellow color)? [SM 9221 B-2014 (3) (b) (2)]			
79	Are sample tubes that show no growth or gas re-incubated for another 24 hours at 35 ± 0.5ºC and re-examined after a total of 48 ± 3 hr? [SM 9221 B-2014 (3) (b) (2)]			
80	Is each tube showing, growth or acidity gently shaken or rotated? [SM 9221 E-2014 (1) (b) (1)]			
81	Is growth from the positive presumptive sample tubes transferred to tubes containing the EC broth using a sterile 3- or 3.5-mm diameter loop or sterile wooden applicator stick? [SM 9221 E-2014 (1) (b) (1)]			Use one or more loopfuls or insert stick at least 2.5 cm into the culture, promptly remove and plunge to the bottom of the fermentation tube containing the broth.
82	Are all EC tubes place in a water bath within 30 minutes of inoculation? [SM 9221 E-2014 (1) (b) (2)]			
83	Are inoculated EC broth tubes incubated at 44.5 ± 0.2ºC for 24 ± 2 hr? [SM 9221 E-2014 (1) (b) (2)]			
84	Is there sufficient water depth maintained in the water bath to immerse tubes to the upper level of the medium? [SM 9221 E-2014 (1) (b) (2)]			
85	Skip to question 85.			
	PROCEDURE – Sample Analysis w/ A-1 Medium	**LAB**	**SOP**	**EXPLANATION**
86	Is the concentration of the A-1 broth used in each series based on the amount of inoculum added? [SM 9221 E-2014 (2) (a)]			For 10mL samples, prepare double-strength medium so the final concentration of ingredients after sample addition is correct.
87	For Class A samples: Is the first series of 5 tubes inoculated with 10 mL of the blender contents? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.2), liquid samples (1) and solid samples (1)]			Prepare the sample as described for "Class B Alternative 1, Liquid Samples *(or Solid Samples),"* except inoculate each of the first series of 5 tubes with 10.0 mL of the blender contents (the concentration of the enrichment broth must be adjusted to compensate for the volume of added sample). [Liquid Samples] This is equivalent to adding 1.0 mL of sludge to the first series of tubes. [Solid Samples] This is equivalent to adding 1.0 g of sludge (wet weight) to the first series of tubes.
88	For Class A samples: Are the remaining series of tubes inoculated using tenfold serial dilutions? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.2), liquid samples (1) and solid samples (1)]			Inoculate at least four series of five tubes using tenfold serial dilutions.
89	For Class B samples: Are five tubes containing 10 ml of double-strength medium inoculated with 10 ml of sample solution "B"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			SM 9221 E-2014 (2)(b) states: Procedure: Inoculate tubes of A-1 broth as directed in 9221B.3b). Incubate for 3 h at 35 ± 0.5°C. Transfer tubes to a water bath at 44.5 ± 02°C and incubate for another 21 ± 2 h. Inoculate the first series of 5 tubes each with 10.0 mL of dilution "B." This is a 0.0010 m L of the original sample.
90	For Class B samples: Are five tubes containing 10 ml of single-strength medium inoculated with 1.0 ml of sample solution "B"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			The second series of tubes should be inoculated with 1.0 mL of dilution "B" (0.00010).

91	For Class B samples: Are five tubes containing 10 ml of double-strength medium inoculated with 10 ml of sample solution "C"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			The third series of tubes should receive 10.0 mL of "C" (0.000010).
92	For Class B samples: Are five tubes containing 10 ml of single-strength medium inoculated with 1.0 ml of sample solution "C"? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (5)]			Inoculate a fourth series of 5 tubes each with 1.0 mL of dilution "C" (0.0000010). Continue the procedure as described in SM.
93	Are all samples incubated at 35 ± 0.5ºC for 3 hr? [SM 9221 E-2014 (2) (b)]			
94	Are tubes then transferred to a water bath and incubated at 44.5 ± 0.2ºC for another 21 ± 2 hrs? [SM 9221 E-2014 (2) (b)]			
	PROCEDURE – Sample Interpretation	**LAB**	**SOP**	**EXPLANATION**
95	Is the MPN index/100 mL for each dilution series calculated according to SM 9221 E-2014? [NC WW/GW LCB Policy]			Only three series of five tubes each will be used for determining the MPN. See the "Fecal Sludge MPN – How to Determine the Appropriate Dilutions when Referencing SM 9221 E-2014" document on page 16 of this document,
96	Is SM 9221C-2014, Table IV used to estimate the MPN index/100 mL? [NC WW/GW LCB Policy] [SM 9221 E-2014 (2) (c)] [SM 9221 C-2014 (2)]			MPN Table IV is on page 18 of this checklist.
97	How are Class A sludge sample results calculated? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (6)] [SM 9221 C-2014 (2)] [NC WW/GW LCB Policy]			$\text{MPN Fecal Coliform/g} = \frac{10 \times \text{MPN Index/100 mL}}{\text{largest volume} \times \% \text{ dry solids}}$ NOTE: % dry solids is **not** in decimal form. NOTE: "largest volume" refers to the volume of the original mixture (30g in 270 mL) that is in the least diluted of the appropriate three dilutions. See the "Fecal Sludge MPN – How to Determine the Appropriate Dilutions when Referencing SM 9221 E-2014" document on page 16 of this document.
98	For Class A sludges, is the result calculated for each sample analyzed? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Section 9.5]			Section 9.5 states: For Class A biosolids, **analytical results are not averaged**: every sample analyzed must meet the Class A requirements.: "Either the density of fecal coliform in the sewage sludge must be less than 1,000 MPN per gram of total solids (dry weight basis), or the density of Salmonella sp. bacteria in sewage sludge must be less than 3 MPN per 4 grams of total solids (dry weight basis)."
99	How are Class B sludge sample results calculated? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Appendix F. (1.1), liquid samples (6)] [NC WW/GW LCB Policy])] [SM 9221 C-2014 (2)]			$\text{MPN Fecal Coliform/g} = \frac{10 \times \text{MPN Index/100 mL}}{\text{largest volume} \times \% \text{ dry solids}}$ NOTE: % dry solids is not in decimal form. NOTE: "largest volume" refers to the volume of the original mixture (30g in 270 mL) that is in in the least diluted of the appropriate three dilutions. See the "Fecal Sludge MPN – How to Determine the Appropriate Dilutions when Referencing SM 9221 E-2014" document on page 15 of this document,
100	For Class B sludges, is the geometric mean calculated using all samples analyzed? [Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, (July 2003) Section 9.5]			
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
101	Are lot numbers of applicable consumable materials			Rule: The laboratory shall have a documented

	documented? [15A NCAC 2H .0805 (a) (7) (K) and NC WW/GW LCB Policy]			system of traceability for the purchase, preparation, and use of all chemicals, reagents, standards, and consumables. NC WW/GW LCB Policy states: All chemicals, reagents, standards and consumables used by the laboratory must have the following information documented: Date received, Date Opened (in use), Vendor, Lot Number, and Expiration Date (where specified). Consumable materials such as pH buffers, lots of pre-made standards and/or media, solids and bacteria filters, etc. are included in this requirement.
102	Is an autoclave log maintained? [SM 9020 B-2015 (4) (h)] and [15A NCAC 2H .0805 (a) (7) (l)]			SM states: Record items sterilized and sterilization temperature along with total run-time (exposure to heat), actual time period at sterilization temperature, set and actual pressure readings, and initials of responsible person for each run cycle. This means three times must be recorded (start time, time it reaches set point and end time). Alternatively, verify the cycle time at operating temperature and pressure annually and document cycle start time and length each day of use.
103	When sterilizing media, is the time that media is actually removed from the autoclave documented to show that it was not exposed to elevated temperature for > 45 minutes after the completion of the sterilization? Ref: [SM 9020 B-2015 (4) (h)]			Do not expose media containing carbohydrates to elevated temperatures for >45 min; some media cannot be exposed to heat for that long. Autoclaving equipment requires the documentation of three times. Autoclaving media requires documentation of four times.
104	Is the autoclave temperature checked weekly with a maximum registering thermometer and documented? [SM 9020 B-2015 Table 9020:I] and [15A NCAC 2H .0805 (a) (7) (E)]			For routine use, verify autoclave temperature weekly with a maximum registering thermometer (MRT) (generally a mercury-filled Teflon-coated device) or accurate high-temperature data logger (HTDL) able to withstand 15-20 lb/in2. If neither device is available, use a strip or pie chart recorder with interpretations written on the chart. Maintain verification records. Must distinguish between daily autoclave temperature and reading from the weekly maximum registering thermometer (MRT) placed inside autoclave in documentation. Annual calibration of the maximum registering thermometer is not required.
105	If glassware is sterilized in an oven, is it at ≥170 ºC for a minimum of 2 hours? [SM 9040-2013]			To sterilize glassware via dry heat, use a hot-air oven set at ≥170 ° C for 2 hours or longer.
106	How are sample bottles sterilized in house? [SM 9020 B-2015 Table 9020: IV] and [SM 9040-2013] **ANSWER:**			Sample bottles may be sterilized in an autoclave at 121ºC for 15 min. For all bottles, loosen caps before autoclaving. If desired after autoclaving, remove moisture present in empty sterile containers by placing items in a drying oven Many labs use disposable commercially sterilized bottles or sample bags.
107	Are laboratory sterilized bottles checked for sterility? [SM 9020 B-2015 (5) (d)]			SM States Test for sterility at least one or a set percentage (e.g., 1 to 4%) of each batch sterilized in the laboratory or of each pre-sterilized lot purchased from a vendor. Need to add sterile dilution/rinse water to bottle and analyze. We will accept Certificate of Analysis for store bought bottles or sample bags in lieu of the above testing.

108	Are the incubator temperatures documented twice daily separated by 4 hours? [SM 9020 B-2015 (4) (n)]			When incubator is in use, (i.e., samples are being incubated), monitor and record corrected temperature twice daily separated by 4 hours.
109	Is the thermometer/temperature monitoring device immersed to the proper depth? ["User-Friendly Guidance on the Replacement of Mercury Thermometers"]			Be sure to check thermometer in water bath to ensure tip is not sitting on bottom of incubator. Check thermometer immersion type (total vs. partial) and line. Thermometers with no indicated depth are the total immersion type. When a partial-immersion thermometer is used, the bottom of the thermometer up to the immersion line should be exposed to the temperature being measured, with the remainder of the thermometer exposed to ambient conditions. When a total immersion thermometer is used, the bulb and the entire portion of the stem containing liquid, except for the last 1 cm, are exposed to the temperature being measured. If the thermometer is not used in this manner, the thermometer immersion is incorrect. Probe type thermometers also have manufacturer specified immersion depths. https://www.epa.gov/sites/production/files/2015-10/documents/nistuserfriendlyguide.pdf
110	Is the temperature-measuring device verified at least quarterly? [15A NCAC 2H .0805 (a) (7) (N) (iii)]			Digital temperature-measuring devices and temperature-measuring devices used in incubators shall be verified at the temperature of use every three months against a Reference Temperature-Measuring Device and their accuracy shall be corrected.
111	Does the temperature-measuring device have an initial stated accuracy of ± 0.1 °C and vary by ≤ 1.0 °C on subsequent checks against the Reference Temperature-Measuring Device? [15A NCAC 2H .0805 (a) (7) (N)] [NC WW/GW LCB Policy]			All temperature-measuring devices shall have accuracy that meets or exceeds one-half the tolerance required for its intended use. All temperature-measuring devices shall be used, stored, and maintained according to the manufacturer's instructions. Therefore, incubators with an incubation temperature tolerance of ± 0.2 °C (e.g., fecal coliform incubators) must have temperature measuring devices with a stated accuracy of ± 0.1 °C. If the temperature-measuring device reading differs from the Reference Temperature-Measuring Device reading by more than 1.0 °C during subsequent verifications, the temperature-measuring device must be replaced.
112	Is the temperature correction posted? [SM 9020 B-2015 4 (a)]			Record accuracy-check results, along with the date, device identification number, and the technician's signature or initials – in a QC logbook. If a correction calculation is necessary, mark the appropriate correction factor on the device so only corrected temperature values are recorded A correction of ± 1.0 °C is the maximum that would be allowed per our Policy.
113	Is a culture positive analyzed with each batch of prepared media or each week for purchased ready-to-use media? [SM 9020 B-2015 (9) (b)] **Indicate which type of media is used (laboratory prepared or commercially prepared):**			SM Table 9020:I. states: media – Check performance with + and - culture controls – Each batch or lot. **Due to the reasons given in question #1, NC WW/GW LCB will require a culture positive (no culture negative) once per week for purchased premade media and once per prepared batch for laboratory prepared media. No culture negative will be required at this time.**

114	Are sterility checks performed on media before first use? [SM 9020 B-2015 (9) (d)]			Test media sterility before first use. Incubate minimally one per lot or a set percentage, e.g., 1 to 4%, of laboratory-prepared and ready-to-use medium, broth, at an appropriate temperature for the amount of time the test would be performed and observe for growth. Record results. If any contamination is indicated, determine the cause and reject analytical data from the samples tested with these materials.
115	Is reagent water testing being performed? [NC WW/GW LCB Policy]			At a minimum, reagent water used to make dilutions, prepare buffered dilution/rinse water or prepare media must be analyzed at least every twelve months for the following parameters: Specific Conductance, Total Organic Carbon, Cadmium, Chromium, Copper, Nickel, Lead, and Zinc. Maximum Acceptable Limits are: Total Organic Carbon < 1.0 mg/L Specific Conductance < 2 µmhos/cm Heavy Metals, single element < 0.05 mg/L Heavy Metals, Total of specified elements < 0.10 mg/L If the facility is using vendor purchased reagent water or dilution/rinse water ,this testing is not required as long as the Certificate of Analysis from the manufacturer meets these requirements and is kept on file.
116	Are at least five percent of all samples analyzed in duplicate to document precision? Or, if analyzing less than 20 samples per month, is at least one duplicate analyzed per month? [15A NCAC 2H .0805 (a) (7) (C)]			Except where otherwise specified in an analytical method, laboratories shall analyze five percent of all samples in duplicate to document precision. Laboratories analyzing fewer than 20 samples per month shall analyze one duplicate during each month that samples are analyzed. **At this time, we will follow our Rules for duplicate frequency.**
117	What is the acceptance criterion for duplicates? [15A NCAC 2H .0805 (a) (7)] and [15A NCAC 2H .0805 (a) (7) (A)] **ANSWER:**			Each laboratory shall have a formal process to track and document review dates and any revisions made in all quality assurance, quality control, and Standard Operating Procedure documents. Supporting Records shall be maintained as evidence that these practices are implemented. Unless specified by the method or this Rule, each laboratory shall establish performance acceptance criteria for all quality control analyses.
118	Does the laboratory analyze a duplicate sample for % solids each day or with each batch of ≤20 samples, whichever is more frequent? [SM 2540 A-2015 (5) and SM 2020 B-2017 Table 2020:II]]			
119	What is the acceptance criterion for % solids duplicates? [15A NCAC 2H .0805 (a) (7) (B)]] **ANSWER:**			Unless specified by the method or this Rule, each laboratory shall establish performance acceptance criteria for all quality control analyses.
120	What corrective action does the laboratory take if the duplicate sample results are outside of established control limits or method precision limits? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible. 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.

The correction below was listed in the Errata for the 22nd edition of Standard Methods. The error was not corrected in the 23rd edition and was mistakenly omitted from the Errata for the 23rd edition.

In Section 9221 C.2 (p. 9-74), in the example near the bottom left of the page, it should say, “Thus, the calculated MPN = **780**/100 mL”.

Additional Comments:

_____
_____
_____
_____
_____
_____
_____

Inspector: _____Date:_____

Fecal Sludge MPN – How to Determine the Appropriate Dilutions when Referencing SM 9221 E-2014

Standard Methods states that when more than three dilutions are used in a decimal series of dilutions, use the guidelines in SM 9221 C-2014 (2) to select the three most appropriate dilutions and refer to Table 9221:IV. We have found that the language there can be confusing because it is interlaced with comments relevant only when using specific dilution schemes. For example, in one place SM states, "If no dilution has all positive tubes (Example F), select the lowest two dilutions, corresponding to 10 and 1 mL sample". This does not mean that your particular dilution scheme must have tubes that contain 10 and 1 mL of sample. Your dilution scheme may be different. This technical assistance document uses the instructions in Standard Methods but does not include those type examples and simply provides the steps that one would go through to narrow the dilutions down to the appropriate three.

Also, be aware that the steps and conditional questions in Standard Methods are being asked with the assumption that you have 5 dilutions. While having 5 dilutions is recommended, a minimum of 4 dilutions is required.

The black text below is language as it appears in Standard Methods with the example references removed. Yellow highlighted text is intended to help explain or clarify what the conditional questions and instructions in black mean.
Proceed through the numbered steps below until you have identified the appropriate three dilutions to use in the final MPN determination. Once you have your three appropriate dilutions, determine the MPN Index/100 mL value from Table 9221:IV. **If the three dilutions selected are not found in Table 9221:IV**, use the highest dilution with at least one positive tube and the two dilutions immediately lower as the three selected dilutions.

1. First, remove the highest dilution (the most dilute, with the least amount of sample) if it has all negative tubes and at least one remaining dilution has a negative tube.

2. Next, remove the lowest dilution (the least dilute, largest sample volume) if it has all positive tubes and at least one remaining dilution has a positive tube.

   - *Remember, these questions are being asked with the assumption that you have 5 dilutions. If both steps 1 and 2 caused the removal of a dilution, that would have brought you down to 3 dilutions and you would be done before getting to Step 3.*

3. If the lowest dilution does not have all positive tubes, and several of the highest dilutions have all negative tubes, then remove the highest negative dilutions.

   - *This question is saying, if Step 2 did not cause the removal of a dilution and any of your highest dilutions (after removal of the all-negative highest dilution, which is Step 1) still have all negative tubes, then remove them*.

4. More than three dilutions may remain after removal of the lowest dilution with all positive tubes and high dilutions with all negative tubes.

   - *This doesn't necessarily mean that your lowest dilution had all positive tubes and you removed it (Step 2) or that your highest dilution had all negative tubes and you removed it (Step 1). It*

could simply mean that your lowest dilution did not have all positive tubes to begin with or that your highest dilution did not have all negative tubes.

In this case, if the highest dilution with all positive tubes is within two dilutions of the highest dilution with any positive tubes, then use the highest dilution with any positive tubes and the two immediately lower dilutions.

- *Remember, in Step 4, you are only looking at the dilutions that remain after having gone through steps 1-3*.

5. If, after removal of the lowest dilution with all positive tubes, no dilution with all positive reactions remains, then select the lowest two dilutions and assign the sum of any remaining dilutions to the third dilution.

- *Again, the language saying "If, after removal of the lowest dilution with all positive tubes, no dilution with all positive reactions remains, …" is talking about Step 2. So what Step 5 is saying is, if the scenario you are looking for in Step 4 (an all positive tube dilution series that is within two dilutions of the highest dilution series with any positive tubes) does not exist, then you use the two lowest dilutions as they are and then add the number of positive tubes in the remaining dilutions together to get your third dilution*.

From there, use the formula in Appendix F, Section 1.1 (6) of *Control of Pathogens and Vector Attraction in Sewage Sludge*, EPA/625/R-92/013, (July 2003), more commonly known as the White House book, to determine the final MPN/gram result. See below.

$$\text{MPN Fecal Coliform/g} = \frac{10 \times \text{MPN Index/100 mL}}{\text{largest volume} \times \%\ \text{dry solids}}$$

NOTE: % dry solids is **not** in decimal form.

NOTE: "largest volume" refers to the volume of the original mixture (30 mL in 300 mL, for aqueous samples; 30 g in 270 mL, for non-aqueous samples) that is in the least diluted of the three dilutions used to determine the MPN value. For example, if dilutions were prepared as directed in the White House book, your dilutions will contain 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mL of the original sample. Since you must use only three dilutions to determine the MPN, the "largest volume" dilution would have to be either 10^{-3} or 10^{-4}.

MPN Index for Various Combinations of Positive Results When Five Tubes Are Used Per Dilution

Recreated from SM 9221C-2014, Table IV

Combination of Positives	MPN Index/100 mL	Combination of Positives	MPN Index/100 mL
0-0-0	<1.8	4-0-3	25
0-0-1	1.8	4-1-0	17
0-1-0	1.8	4-1-1	21
0-1-1	3.6	4-1-2	26
0-2-0	3.7	4-1-3	31
0-2-1	5.5	4-2-0	22
0-3-0	5.6	4-2-1	26
1-0-0	2.0	4-2-2	32
1-0-1	4.0	4-2-3	38
1-0-2	6.0	4-3-0	27
1-1-0	4.0	4-3-1	33
1-1-1	6.1	4-3-2	39
1-1-2	8.1	4-4-0	34
1-2-0	6.1	4-4-1	40
1-2-1	8.2	4-4-2	47
1-3-0	8.3	4-5-0	41
1-3-1	10	4-5-1	48
1-4-0	10	5-0-0	23
2-0-0	4.5	5-0-1	31
2-0-1	6.8	5-0-2	43
2-0-2	9.1	5-0-3	58
2-1-0	6.8	5-1-0	33
2-1-1	9.2	5-1-1	46
2-1-2	12	5-1-2	63
2-2-0	9.3	5-1-3	84
2-2-1	12	5-2-0	49
2-2-2	14	5-2-1	70
2-3-0	12	5-2-2	94
2-3-1	14	5-2-3	120
2-4-0	15	5-2-4	150
3-0-0	7.8	5-3-0	79
3-0-1	11	5-3-1	110
3-0-2	13	5-3-2	140
3-1-0	11	5-3-3	170
3-1-1	14	5-3-4	210
3-1-2	17	5-4-0	130
3-2-0	14	5-4-1	170
3-2-1	17	5-4-2	220
3-2-2	20	5-4-3	280
3-3-0	17	5-4-4	350
3-3-1	21	5-4-5	430
3-3-2	24	5-5-0	240
3-4-0	21	5-5-1	350
3-4-1	24	5-5-2	540
3-5-0	25	5-5-3	920
4-0-0	13	5-5-4	1600
4-0-1	17	5-5-5	>1600
4-0-2	21		

Appendix F
Sample Preparation for Fecal Coliform Tests and *Salmonella* sp. Analysis

1. Sample Preparation for Fecal Coliform Tests

1.1 Class B Alternative 1

To demonstrate that a given domestic sludge sample meets Class B Pathogen requirements under alternative 1, the density of fecal coliform from at least seven samples of treated sewage sludge must be determined and the geometric mean of the fecal coliform density must not exceed 2 million Colony Forming Units (CFU) or Most Probable Number (MPN) per gram of total solids (dry weight basis). The solids content of treated domestic sludge can be highly variable. Therefore, an aliquot of each sample must be dried and the solids content determined in accordance with procedure 2540 G. of the 18th edition of Standard Methods for the Examination of Water and Wastewater (SM).

Sludge samples to be analyzed in accordance with SM 9221 E. Fecal Coliform MPN Procedure and 9222 D. Fecal Coliform Membrane Filter Procedure may require dilution prior to analysis. An ideal sample volume will yield results which accurately estimate the fecal coliform density of the sludge. Detection of fecal coliform in undiluted samples could easily exceed the detection limits of these procedures. Therefore, it is recommended that the following procedures be used (experienced analysts may substitute other dilution schemes as appropriate).

For Liquid Samples:

1. Use a sterile graduated cylinder to transfer 30.0 mL of well mixed sample to a sterile blender jar. Use 270 mL of sterile buffered dilution water (see Section 9050C) to rinse any remaining sample from the cylinder into the blender. Cover and blend for two minutes on high speed. 1.0 mL of this mixture is 0.1 mL of the original sample or $1.0X10^{-1}$.

2. Use a sterile pipette to transfer 11.0 mL of the blended sample mixture to 99 mL of sterile buffered dilution in a sterile screw cap bottle and mix by vigorously shaking the bottle a minimum of 25 times. This is dilution "A." 1.0 mL of this mixture is 0.010 mL of the original sample or $1.0X10^{-2}$.

3. Use a sterile pipette to transfer 1.0 mL of dilution "A" to a second screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "B." 1.0 mL of this mixture is 0.00010 mL of the original sample or $1.0X10^{-4}$.

4. Use a sterile pipette to transfer 1.0 mL of dilution "B" to a sterile screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "C." Go to step 5 for MPN analysis (preferred) or 7 for MF analysis.

5. For MPN analysis, follow procedure 9221 E. in SM. Four series of 5 tubes will be used for the analysis. Inoculate the first series of 5 tubes each with 10.0 mL of dilution "B." This is a 0.0010 mL of the original sample. The second series of tubes should be inoculated with 1.0 mL of dilution "B" (0.00010). The third series of tubes should receive 10.0 mL of "C" (0.000010). Inoculate a fourth series of 5 tubes each with 1.0 mL of dilution "C" (0.0000010). Continue the procedure as described in SM.

6. Refer to Table 9221.IV. in SM to estimate the MPN index/100 mL. Only three of the four series of five tubes will be used for estimating the MPN. Choose the highest dilution that gives positive results in all five tubes, and the next two higher dilutions for your estimate. Compute the MPN/g according to the following equation:

$$\text{MPN Fecal Coliform/g} = \frac{10 \times \text{MPN Index/100 mL}}{\text{largest volume} \times \%\ \text{dry solids}}$$

Examples:

In the examples given below, the dilutions used to determine the MPN are underlined. The number in the numerator represents positive tubes; that in the denominator, the total number of tubes planted; the combination of positives simply represents the total number of positive tubes per dilution.

Example	0.0010 mL	0.00010 mL	0.000010 mL	0.0000010 mL	Combination of positives
a	5/5	5/5	3/5	0/5	5-3-0
b	5/5	3/5	1/5	0/5	5-3-1
c	0/5	1/5	0/5	0/5	0-1-0

$$\text{coliform colonies/g} = \frac{(2+18)\times 100}{(0.000010 + 0.00010) \times 4.3} = 4.2 \times 10^6$$

Sample number 3 has two filters which have colony counts outside the ideal range also. In this case both countable plates should be used to calculate the coliform density/g. For sample number 3, the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{(8 + 65) \times 100}{(0.00010 + 0.0010) \times 4.0} = 1.6 \times 10^6$$

Except for sample number 5, all of the remaining samples have at least one membrane filter within the ideal range. For these samples, use the number of colonies formed on that filter to calculate the coliform density. For sample number 1, the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{23\times 100}{0.0010 \times 3.8} = 6.0 \times 10^5$$

Coliform densities of all the samples were calculated and converted to $\log_{10}$ values to compute a geometric mean. These calculated values are presented in Table 2.

Table 2. Coliform Density of Sludge Samples

Sample No.	Coliform Density	$\log_{10}$
1	6.0×10^5	5.78
2	4.2×10^6	6.63
3	1.6×10^6	6.22
4	1.4×10^5	6.14
5	4.0×10^5	5.60
6	1.0×10^6	6.02
7	5.1×10^5	5.71

The geometric mean for the seven samples is determined by averaging the $\log_{10}$ values of the coliform density and taking the antilog of that value.

$(5.78 + 6.63 + 6.22 + 6.14 + 5.60 + 6.02 + 5.71)/7 = 6.01$

The antilog of $6.01 = 1.03 \times 10^6$

Therefore, the geometric mean fecal coliform density is below 2 million and the sludge meets Class B Pathogen requirements under alternative 1.

1.2 Class A Alternative 1

Part 503 requires that, to qualify as a Class A sludge, treated sewage sludge must be monitored for fecal coliform (or *Salmonella* sp. and have a density of less than 1,000 MPN fecal coliform per gram of total solids (dry weight basis). The regulation does not specify total number of samples. However, it is suggested that a sampling event extend over two weeks and that at least seven samples be collected and analyzed. The membrane filter procedure may not be used for this determination. This is because the high concentration of solids in such sludges may plug the filter or, render the filter uncountable. The total solids content for each sample must be determined in accordance with procedure 2540 G. of SM.

For Liquid Samples:

1. Follow procedure 9221 E. in SM. Inoculate at least four series of five tubes using ten fold serial dilutions. Prepare the sample as described for "Class B Alternative 1, Liquid Samples," except inoculate each of the first series of tubes with 10.0 mL of the blender contents (the concentration of the enrichment broth must be adjusted to compensate for the volume of added sample). This is equivalent to adding 1.0 mL of sludge to the first series of tubes. Inoculate the remaining tubes and complete the analysis in accordance with SM.
2. Calculate the MPN as directed in Step 4 above.

For Solid Samples:

1. Follow procedure 9221 E. in SM. Inoculate at least four series of five tubes using ten fold serial dilutions. Prepare the sample as described for "Class B Alternative 1, Solid Samples," except inoculate each of the first series of tubes with 10.0 mL of the blender contents (the concentration of the enrichment broth must be adjusted to compensate for the volume of added sample). This is equivalent to adding 1.0 g of sludge (wet wieght) to the first series of tubes. Inoculate the remaining tubes and complete the analysis in accordance with SM.
2. Calculate the MPN as directed in step 4 above.

2. Sample Preparation for *Salmonella* sp. Analysis

Salmonella sp. quantification may be used to demonstrate that a sludge meets Class A criteria, instead of analyzing for fecal coliforms. Sludges with *Salmonella* sp. densities below 3 MPN/4 g total solids (dry weight basis) meet Class A criteria. The analytical method described in Appendix F of this document describes the procedure used to identify *Salmonella* sp. in a water sample. Similarly, the procedures for analysis of *Salmonella* sp. in SM (Section 9260 D) do not address procedures for sludges, the sample preparation step described here should be used, and the total solids content of each sample must be determined according to method 2540 G in SM.

For Liquid Samples:

1. Follow the same procedure used for liquid sample preparation for fecal coliform analysis described under "Class A Alternative 1." However, the enrichment medium used for this analysis should be dulcitol selenite broth (DSE) as described in Appendix G of this document or dulcitol selenite or tetrathionate broth as described in SM. Only three series of five tubes should be used for this MPN procedure. Use a sterile open tip pipette to transfer 10.0 mL of well mixed sample to each tube in the first series. These tubes should contain 10.0 mL of double strength enrichment broth. Each tube in the second series should contain 10.0 mL of double strength enrichment broth. These tubes should each receive 10.0

mL of the blended mixture. The final series of tubes should contain 10.0 mL of single strength enrichment broth. These tubes should each receive 1.0 mL of the blended mixture. Complete the MPN procedure as described in Appendix G or SM as appropriate.

2. Refer to Table 9221.IV. in SM to estimate the MPN index/100 mL. Calculate the MPN/4 g according to the following equation:

$$\textit{Salmonella}\text{ sp. MPN/4 g} = \frac{\text{MPN Index/100 mL x 4}}{\text{\% dry solids}}$$

For example:

If one tube in the first series was identified as being positive for *Salmonella* sp. and no other tubes were found to be positive, from Table 9221.IV one finds that a 1-0-0 combination of positives has an MPN index/100 mL of 2. If the percent of dry solids for the sample was 4.0, then:

$$\textit{Salmonella}\text{ sp. MPN/4g} \qquad \frac{2 \times 4}{4.0 = 2} = 2$$

For Solid Samples:

1. Follow the procedure for solid sample preparation for fecal coliform analysis described under Class A Alternative 1 above. However, the enrichment medium used for this analysis should be dulcitol selenite broth (DSE) as described in Appendix G or dulcitol selenite or tetrathionate broth as described in SM, and only three series of five tubes should be used for this MPN procedure. Use aseptic technique to weigh out and transfer 10.0 g of well mixed sample to each screw cap tube in the first series, shake vigorously to mix. These tubes should contain 10.0 mL of double strength enrichment broth. Likewise, each tube in the second series should contain 10.0 mL of double strength enrichment broth. These tubes should receive 10.0 mL of the blended mixture. The final series of tubes should contain 10.0 mL of single strength enrichment broth. These tubes should receive 1.0 mL of the blended mixture. Alternately, because the calculated detection limit is dependent upon the total solids content of the sample, samples with total solids contents >28% can be blended as described above and the blender contents can be used for inoculating the initial series of tubes. When this option is chosen, the final series of tubes will contain 0.1 mL of the blender contents. Complete the MPN procedure as described in Appendix G or SM as appropriate.

2. Refer to Table 9221.IV. in SM to estimate the MPN index/100 mL. Calculate the MPN/4 g according to the following equation:

$$\textit{Salmonella}\text{ sp. MPN/4g} = \frac{\text{MPN Index/1 00mL x 4}}{\text{\% dry solids}}$$