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: **Thermotolerant (Fecal) Coliform - Membrane Filtration Procedure**
Method: **SM 9222 D-2015 (MF) (Aqueous)**

EQUIPMENT: Not all items are required.

	Water bath incubator, 44.5 ± 0.2ºC (calibrated or accurate to ± 0.1°C accuracy) with gabled cover		Autoclave
	pH meter with solid surface probe for agar		Temperature gauge
	Thermometer 0.1°C increments		• Pressure gauge
	Graduated cylinders		• Holding thermometer
	Sterile pipettes		• Vacuum source
	Refrigerator		Sterile non-leaking filtration apparatus
	Microscope w/ 10-15x magnification or other optical device		Colony counter
	Forceps		Verification equipment
	Sterilizer oven 170ºC		Incubator, 35.0 ±0.5°C (air or water)
	Hotplate w/ magnetic stirrer		• Fermentation tubes
	Maximum Registering Thermometer		• Durham tubes
	Bunsen Burner (or flame source) with alcohol to flame		• Inoculating equipment
	Dilution bottles		

CONSUMABLES: Not all items are required

	Waterproof plastic bag enclosures		Petri dishes
	Sterile absorbent pads		Sterile membrane filters, 0.45 µm

REAGENTS: Not all items are required

	M-FC broth		Sterile dilution/ rinse water
	M-FC agar		• Phosphate buffer (KH_2PO_4)
	1% Rosolic Acid		• $MgCl_2$
	0.2 *N* NaOH		Lauryl Tryptose Broth (LTB)
	10% $Na_2S_2O_3$		EC Medium

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)] **ANSWER:**			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. **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. 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	**LAB**	**SOP**	**EXPLANATION**
4	Are samples collected in sterile containers? [SM 9060 A-2013 (1) and 40 CFR Part 136.3 Table II]			Collect samples for microbiological examination in clean, sterile, nonreactive borosilicate glass or plastic bottles (i.e., any plastic that is made of a sterilizable material such as polypropylene or other autoclavable plastic) or presterilized plastic bags appropriate for microbiological use. Sterilize as directed in section 9030 B-2015 (19) and 9040-2013.
5	Is residual chlorine neutralized at time of sample collection with sterile $Na_2S_2O_3$? [SM 9060 A-2013 (2) and 40 CFR Part 136.3 Table II]			For sampling chlorinated wastewater effluents, add sufficient $Na_2S_2O_3$ to a clean sample bottle to give a concentration of 100 mg/L in the sample. In a 120-ml bottle 0.1 ml of a 10% solution of $Na_2S_2O_3$ will neutralize a sample containing 15 mg/L residual chlorine. This will yield a **0.008%** $Na_2S_2O_3$ solution as required by 40 CFR Part 136.3 Table II.
6	Are samples iced to < 10 ºC during shipment? [40 CFR 136.3 Table II]			40 CFR Part 136.3 Table II, 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.
7	Are samples checked for residual chlorine upon receipt in the lab? [40 CFR 136.3 Table II]			Use of TRC strips is allowed, see "Required Documentation for Sample Preservation and Hold Time Policy (10/30/2014)". Mix thoroughly prior to checking for chlorine.
8	What action is taken if chlorine is present? [15A NCAC 2H .0805 (a) (7) (M)] **ANSWER:**			If another sample cannot be collected, dechlorinate the sample and notify NC WW/GW Laboratory Certification group that a non-compliant sample was received. Reported results must be qualified.
9	Are samples stored at < 10 ºC prior to analysis? [40 CFR 136.3 Table II]			
	MEDIA	**LAB**	**SOP**	**EXPLANATION**
10	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.
11	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 S.U. 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.
12	Is the stock phosphate buffer documented to be pH 7.2 ± 0.5 S.U.? This is considered pertinent information. [SM 9050 C-20015 (1) (a) (1)] and [15A NCAC 2H .0805 (a) (7) (E)]			If prepared, document in the preparation instructions or if purchased, retain manufacturer's documentation stating it is the proper pH. All analytical records, including original observations and information necessary to facilitate historical reconstruction of the calculated results, shall be maintained for five years. All analytical data and records pertinent to each certified analysis shall be available for inspection upon request.
13	Is the M-FC media purchased pre-made and ready to use or prepared in the lab? If purchased pre-made skip to question #20. **ANSWER:**			Prepare according to manufacturer's instructions: Suspend 3.7 grams of the powder in 100 mL of purified water. Add 1 mL of a 1% solution of Rosolic Acid in 0.2 N NaOH> If necessary adjust the pH to 7.4 with 1 N HCl. Heat to boiling. Do not autoclave. Cool before use. Note: For most samples, mFC medium may be used without the 1% Rosolic acid addition, provided there is no interference with background growth. Ref: 9222 D-2006 (1) (a).
14	If <u>prepared in the lab</u>, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 B (5) (j) (1) Page 9-17states: Document preparation activities such as name of medium, volume produced, format, final pH, date prepared, and name of preparer.
15	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)]			
16	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
17	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.
18	Is pH of the M-FC medium adjusted if necessary and documented to be 7.4 ± 0.2 S.U.? [SM 9222 D-2015 (2)] and [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) states: After sterilization, check and record pH of a portion of each medium because the specified pH of the medium is the actual pH required for adequate growth. If pH adjustment is needed, use filter-sterilized 1*N* NaOH or 1*N* HCl solutions to make minor adjustments so medium's pH meets that specified in the formulation. (Commercially available media will seldom need pH adjustment). If medium is known to require pH adjustment, adjust it appropriately before sterilization and record final pH. If the pH difference is >0.5 units, discard the batch and check both preparation instructions and reagent water's pH to resolve the problem.

				SM 9222 D-2015 (2) states: Final pH should be 7.4 ± 0.2 S.U. Bottom line: It is required to check and document the pH of each batch of prepared media after sterilization. If the pH is not 7.4 ± 0.2 S.U. it must be adjusted to that range. If using agar, the method states to pour into petri plates and let solidify before it talks about the final pH. Therefore, they need to use a pH electrode for surface measurements, which is made to be used for solid and semi-solid samples, and analyze the agar after it solidifies.
19	What is the holding time for the prepared media? [SM 9222 D-2006 (2)] **ANSWER:**			Discard unused broth after 96 h or unused agar after 2 weeks.
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 states: For prepared ready-to-use media with a manufacturer's expiration date greater than noted in the Table, have manufacturer supply evidence of media quality for that extended period of time. 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.
21	Is media stored in refrigerator in the dark? [SM 9222 D-2015 (2)]			SM states: Refrigerate finished medium (preferably in sealed plastic bags or other containers to reduce moisture loss) and discard unused broth after 96 h or unused agar after 2 weeks. Purchased ready-to-use media may be used until the manufacturer's expiration date. See question #20.
22	Are absorbent pads used in conjunction with M-FC medium? [SM 9222 D-2015 (3) (c)] If not, skip to Question #24.			
23	While in the culture dish, is the pad saturated with at least 2.0 ml of M-FC medium and the excess decanted from the dish? [SM 9222 D-2015 (3) (c)]			Not applicable if using agar.
24	Is agar used? [SM 9222 D-2015 (3) (c)]			
25	Is the agar preparation documented? [SM 9020 B-2015 (5) (j) (1)]			SM 9020 B-2015 (5) (j) (1) Page 9-17 states: Document preparation activities such as name of medium, volume produced, format, final pH, date prepared, and name of preparer.
	VERIFICATION MEDIA	**LAB**	**SOP**	**EXPLANATION**
26	Is the LTB medium purchased ready-to-use or prepared in the lab? If purchased ready-to-use skip to question #38.			Although SM 9221 B-2014 (3) (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.
27	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			See explanation in question 25.
28	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)]			
29	Is reagent grade water used in preparing media? [SM			

	9020 B-2015 (5) (j) (1)]			
30	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.
31	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-in fermentation tubes with an inverted vial (Durham tube)-sufficient medium to cover the inverted vial at least one-half to two-thirds after sterilization. Note: Medium will fill the inverted vial when sterilized. Account for this volume when dispensing media into tubes.
32	If a Durham tube is omitted, is 0.01 g/L 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.
33	Is LTB made using 35.6 g/L dehydrated LTB? [SM 9221 B-2014 (3) (a)]			Prepare in accordance with Table 9221:I. NOTE: Since sample is not added to the LTB (the loop is simply dipped in it) only the 1 ml inoculum instructions apply for verification. TABLE 9221:I. PREPARATION OF LAURYL TRYPTOSE BROTH Inoculum mL / Amount of Medium in Tube mL / Volume of Medium + Inoculum mL / Dehydrated Lauryl Tryptose Broth Required g/L 1 / 10 or more / 11 or more / 35.6 10 / 10 / 20 / 71.2 10 / 20 / 30 / 53.4 20 / 10 / 30 / 106.8 100 / 50 / 150 / 106.8 100 / 35 / 135 / 137.1 100 / 20 / 120 / 213.6
34	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. Note: Cap tubes loosely and set autoclave exhaust to slow.
35	After sterilization, are inverted vials free of air bubbles? [SM 9221 B-2014 (3) (a)]			Ensure that inverted vials, if used, are free of air bubbles.
36	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 9221 B-2014 (3) (a) states: Medium pH should be 6.8 ± 0.2 after sterilization. It is required to check and document the pH of each batch of prepared media after sterilization. If the pH is not 6.8 ± 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).
37	Is the EC medium purchased ready-to-use or prepared in the lab? If purchased ready-to-use skip to question #46.			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.
38	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			

39	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)]			
40	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
41	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.
42	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.
43	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. Note: Cap tubes loosely and set autoclave exhaust to slow.
44	After sterilization, are inverted vials free of air bubbles? [SM 9221 E-2014 (1) (a)]			Ensure that inverted vials are free of air bubbles.
45	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 9221 E-2014 (1) (a) states: Medium pH should be 6.9 ± 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 6.9 ± 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 whey (e.g., incorrect preparation or abnormal pH of reagent water).
46	When prepared in-house, are the LTB and EC media used within the holding times specified in Table 9020:V? [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. NOTE: For dehydrated media, you may follow the manufacturer's instructions for preparation; however, you must follow Table 9020: V for hold times.
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 states: For prepared ready to use media with a manufacturer's expiration date greater than noted in the Table, have manufacturer supply evidence of media quality for that extended period of time.
	PROCEDURE	**LAB**	**SOP**	**EXPLANATION**
48	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 22]			Sample analysis should begin as soon as possible after receipt; sample **incubation (not filtration) must be started no later than 8 hours from time of collection.**
49	Are sample volumes selected to yield counts between 20 and 60 colonies per filter? [SM 9222 D-2015 (3) (a)]			
50	Are samples adequately homogenized prior to analysis or dilution? [SM 9060 A-2013 (3) and SM 9222 D-2015 (3) (b)]			Bottles used for sample collection should be large enough to collect desired sample volume and still maintain adequate headspace (2.5 cm) to ensure proper sample mixing (via shaking) before analyses. Thoroughly mix sample or dilution(s) of sample by vigorously shaking (e.g., 25 times up and down in a 1 ft arc in 7 s) to break up clumps of bacteria, which is

				crucial for a microbial quantitative method. If sample bottle lacks enough headspace for adequate mixing, pour sample into a larger sterile vessel to mix appropriately.
51	Are at least two dilutions analyzed? [SM 9222 D-2015 (3) (a)]			Select volume of water sample to be examined in accordance with the information in Table 9222:IV. Use sample volumes that will yield counts between 20 and 60 thermotolerant coliform colonies per membrane. When the sample's bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate. Estimate the volume and/or dilution expected to yield a countable membrane, and select two additional quantities representing one-tenth and ten times (or one third and three times) this volume, respectively.
52	How are sample volumes measured? [SM 9020 B-2015 (4) (k)] and [SM 9030 B-2015 (9)] **ANSWER:**			Pipets, pipettors, graduated cylinders, or graduation marks on the filter funnel are acceptable. If pipet tips are chipped, replace. If using volumetric graduation marks on the filter funnel to measure sample volume, check accuracy of graduation marks initially using a Class A graduated cylinder or volumetric pipet. Record results. Ref: SM 9020 B-2015 (4)(k)
53	If reusable glassware is used, is the glassware checked for pH and inhibitory residues? [SM 9020 B-2015 (5) (a) (1) and (2)] **Not required at this time.**			
54	Are sterile forceps used to place the sterile membrane filter (grid side up) on the filter plate? [SM 9222 D-2015 (3) (b) and SM 9222 B-2015 (4) (c)]			Forceps are sterilized by alcohol flaming. SM 9222 D-2015 (3) (b) refers back to the Total Coliform method - 9222 B-2015 (4) (c) for the filtration of samples.
55	Is the sample filtered under partial vacuum? [SM 9222 D-2015 (3) (b) and SM 9222 B-2015 (4) (c)]			
56	Is the time sample filtration begins documented? This is considered pertinent information. [15A NCAC 2H .0805 (a) (7) (E)]			Needed to determine if samples are put in the incubator within 30 minutes. Need to document three times – beginning (filtration) and in incubator [these will show no more than 30 minutes elapsed between filtration and the start of incubation], and out of incubator [this will document the 24 ± 2-hour incubation time] – see questions # 62 and 64.
57	With the filter still in place, is the interior surface of the filter funnel rinsed with three 20-30 ml portions of sterile buffered dilution water? [SM 9222 D-2015 (3) (b) and SM 9222 B-2015 (4) (c)]			A steady flow of sterile buffered dilution water from of squeeze bottle is acceptable as long as it has been sterilized along with water and does not become contaminated during use – cover tip of bottle with aluminum foil prior to sterilization.
58	Is the prepared filter aseptically placed directly on the agar or pad with a rolling motion to avoid entrapment of air? [SM 9222 D-2015 (3) (b) and SM 9222 B-2015 (4) (c)]			
59	Are prepared dishes sealed in waterproof plastic bags and completely submerged upside down in a water bath? [SM 9222 D-2015 (3) (d)]			Place all prepared dishes in waterproof plastic bags, remove as much air as possible seal, invert and submerge petri dishes in water bath. Anchor dishes below water surface to maintain critical temperature requirements. Do not submerge plates in waterproof hard-sided plastic containers; the extra air space does not allow the plates to reach temperature for many hours
60	Are anchor devices prewarmed before use with samples? [SM 9222 D-2015 (3) (d)]			If anchor devices (e.g., "O" rings. Bricks, or water bottles) will also be submerged, make sure they are prewarmed before sample use, small enough to maintain critical temperature requirements, and do not interfere with sample incubation

61	Are all prepared samples placed in the incubator within 30 minutes of filtration? [SM 9222 D-2015 (3) (d)]			Place all prepared cultures in the water bath within 30 minutes after filtration. To meet the need for greater temperature control use a water bath, a heat sink incubator, or a properly designed and constructed incubator to give equivalent results.
62	Is the time samples are placed in 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
63	Are samples incubated at 44.5 ± 0.2ºC? [SM 9222 D-2015 (3) (d)]			Incubate for 24 ± 2 h at 44.5 ± 0.2ºC.
64	Is the time samples are removed from 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.
65	Are appropriate colonies counted? [SM 9222 D-2015 (3) (e)]			Colonies produced by thermotolerant coliform bacteria on mFC medium are various shades of blue. Non-thermotolerant coliform colonies are gray to cream-colored. Normally, few non-thermotolerant coliform colonies will be observed on mFC medium because the elevated temperature and addition of rosolic acid salt reagent selects against them.
66	How is the density of CFU calculated? [SM 9222 D-2015 (4) (a) and SM 9222 B-2015 (5)] **ANSWER:**			CFU/100ml = $\frac{\text{coliform colonies counted x 100}}{\text{mL sample filtered}}$
67	Are results reported according to the NC WW/GW LC Fecal Coliform Reporting policy document?			See Division document on how to calculate data on the DMR at: http://deq.nc.gov/about/divisions/water-resources/water-resources-permits/wastewater-branch/npdes-wastewater/forms-documents
	COLONY VERIFICATION	**LAB**	**SOP**	**EXPLANATION**
68	Are at least 10 blue colonies verified per month? [40 CFR 136.3 Table IA; footnote 29]			On a monthly basis, at least ten blue colonies from positive samples must be verified using Lauryl Tryptose Broth and EC Broth, followed by count adjustment based on these results; and representative non-blue colonies should be verified using Lauryl Tryptose Broth. Where possible, verification should be done from randomized sample sources. If samples do not routinely produce 10 or more blue colonies, verify the first sample during the month which produces blue colonies, regardless of the number. Adjust sample result accordingly. If no samples during the month produce plates with blue colonies, verify 10 colonies from the culture positive. Count adjustments from the culture positive are not to be applied to sample results. **See the Colony Verification Technical Assistance document attached to the end of this checklist.**
69	Are ten LTB fermentation tubes inoculated with ten blue fecal coliform colonies from a single sample? [SM 9020 B-2015 (10) (b) (2)]			Colonies must be from a single sample but may be collected from multiple filters from that sample.
70	Is the inoculating instrument sterilized between each colony/inoculation? [SM 9222 B-2015 (4) (f) (1)]			
71	Are LTB fermentation tubes incubated at 35 ± 0.5°C for 24 ± 2 hours? [SM 9221 B-2014 (3) (b) (2)]			
72	If a water bath is used, is sufficient water depth maintained to immerse LTB tubes to the upper level of			

	the medium? [SM 9221 E-2014 (1) (b) (2)]			
73	After incubation, is each LTB tube swirled gently and examined for growth and/or gas production and the results documented? [SM 9221 B-2014 (3) (b) (2)]			After 24 ± 2 h swirl each tube or bottle gently and examine it for growth, gas, and/or acidic reaction (shades of yellow color) and, if no gas or acidic reaction is evident, reincubate and reexamine at the end of 48 ± 3 h. Record presence or absence of growth, gas, and/or acid production. If the inner vial is omitted, growth with acidity (yellow color) signifies a positive presumptive reaction.
74	If no gas is evident, are LTB tubes re-incubated at 35 ± 0.5°C for an additional 24 hours and re-examined for growth or gas production after a total of 48 ± 3 hours? [SM 9221 B-2014 (3) (b) (2)]			
75	For any LTB tubes that exhibit growth and/or gas production, are those inoculated into EC medium? [SM 9221 E-2014 (1) (b) (1)]			Gently shake or rotate fermentation tubes or bottles showing gas, growth, or acidity. Using a sterile 3- or 3.5-mm-diam loop or sterile wooden applicator stick, transfer growth from each presumptive or confirmed fermentation tube or bottle to EC broth (see Section 9221B.3).
76	Are EC fermentation tubes incubated within 30 minutes of inoculation? [SM 9221 E-2014 (1) (b) (2)]			Place all EC tubes in a water bath within 30 min after inoculation.
77	Are EC tubes incubated in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours? [SM 9221 E-2014 (1) (b) (2)]			Incubate inoculated EC broth tubes in a water bath at 44.5 ± 0.2°C for 24 ± 2 h.
78	Is sufficient water depth maintained in the waterbath incubator to immerse EC tubes to the upper level of the medium? [SM 9221 E-2014 (1) (b) (2)]			Maintain sufficient water depth in the water bath incubator to immerse tubes to the upper level of the medium.
79	After incubation, is each EC tube examined for growth and gas production and the results documented? [SM 9221 E-2014 (1) (c)]			
80	Are LTB and EC tubes inoculated sequentially at the same time from a single colony to reduce the time of verification analysis? [SM 9222 D-2015 (3) (f)]			
81	When LTB and EC tubes are inoculated at the same time, and only the LTB tubes produce gas, are fresh tubes of EC medium inoculated from the LTB growth and incubated for an additional 24 ± 2 hours?			Based upon EPA Region 4 guidance.
82	If any blue colonies tested do not produce gas, is the colony count of the plate adjusted by the percentage of negative EC medium fermentation tubes prior to reporting results? [40 CFR 136.3 Table IA; footnote 29]			On a monthly basis, at least ten blue colonies from positive samples must be verified using Lauryl Tryptose Broth and EC Broth, followed by count adjustment based on these results; and representative non-blue colonies should be verified using Lauryl Tryptose Broth. Where possible, verification should be done from randomized sample sources. For example, if one of ten EC tubes are negative, multiply colony count of the plate by 90% (0.90) and report the result rounded to whole numbers. If a laboratory finds a low percentage of verification with a certain water supply or matrix, another test method must be chosen. [SM 9020 B-2015 (10)]
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
83	Is a consumables test performed whenever new media, pads or membrane filters are put into use? [NC WW/GW LC Policy]			Before a new lot of consumable materials are used for the Fecal Coliform MF method, those materials must be tested and compared to those currently in use to ensure they are reliable. Consumable materials included in this requirement are: membrane filters and/or pads (often packaged together) and media. It is recommended that only one consumable be tested at a time. At a minimum, make single analyses on five positive samples that will yield 20-60 colonies for both the current lot and the new lot. There are two options for determining acceptance of results: Option 1:

				Follow the acceptance criteria described in Standard Methods 9020 B-2015 5. (*f)* (2) (a) and (b). Option 2: Compare the average colony count of each five-sample set and evaluate against your routine sample duplicate acceptance criterion. This is not required for reagent water.
84	Is comparability data on file to show that results obtained by the membrane filter method for chlorinated effluents are comparable to those obtained with the multiple tube method? [SM 9222 D-2015] **Not required at this time.**			Since the MF test has been the primary regulatory method used for years in NC this will not be required at this time. Also, with the current discharge limits for TRC in place at most NPDES facilities, TRC is generally not found at levels that would significantly impact the MF test. This decision is also based upon the language found in 9020 regarding costs and time required, 40 CFR 136.3 Table IA footnote #5 states: Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.
85	Are lot numbers of applicable consumable materials documented? [15A NCAC 2H .0805 (a) (7) (K) and NC WW/GW LC Policy]			This includes media, filters, pads and dishes. NC WW/GW LC 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.
86	Are short-wave Ultra-Violet (UV) lamps (254 nm) used to sanitize equipment between filtrations? [SM 9020 B-2015 (4) (l) (1)] If not, skip to question 90.			As an alternative to rinsing, to sanitize funnels between samples after filter removal, expose all surfaces of previously cleaned and sterilized assembly to UV radiation for 2 min before reusing units for successive filtrations.
87	Are UV lamp bulbs cleaned monthly with a soft cloth moistened with 70% ethanol? [SM 9020 B-2015 (4) (l) (1)]			Ultraviolet lamps: Disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol.
88	Are UV lamp bulbs tested quarterly with an appropriate UV light meter? [SM 9020 B-2015 (4) (l) (1)]			Test lamps quarterly with an appropriate UV light meter.
89	Are UV lamp bulbs replaced if the output is less than 70% of the original? [SM 9020 B-2015 (4) (l) (1)]			Replace bulbs if the output is less than 70% of the original.
90	Is equipment sterilized in an autoclave? If not, skip to question 93.			
91	Is an autoclave log maintained? [SM 9020 B-2015 (4) (h)] and [15A NCAC 2H .0805 (a) (7) (l)]			SM states: After each run cycle, record the items sterilized, sterilization temperature, total run time (heat exposure), programmed/preset sterilization period, actual pressure readings, and analyst initials. 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.
92	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)]			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. 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.
93	If glassware is sterilized in an oven, is it at 170ºC 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.
94	How is sterilized equipment stored? [SM 9020 B-2015 (5) (a)]			SM 9020 B-2015 (5) (a)states: Either cover glassware or store glassware with its bottom up to prevent dust from settling inside it. Funnels and graduated cylinders may be covered in aluminum foil prior to sterilization for storage until used.
95	How are sample bottles sterilized? [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.
96	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. Autoclave tape alone not adequate – 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.
97	Is the incubator temperature 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.
98	Is the thermometer/temperature monitoring device graduated in 0.1°C increments? [SM 9030 B-2015 (12)]			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.
99	Is the thermometer/temperature monitoring device verified quarterly? [15A NCAC 2H .0805 (a) (7) (N) (iii)]			Rule: 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.
100	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.
101	Is a culture positive analyzed with each batch of prepared media? Each week for purchased ready-to-use media? [SM 9020 B-2015 (9) (b)] [NC WW/GW LC Policy]			SM Table 9020:I. states: media – Check performance with + and - culture controls – Each batch or lot 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.

				SM 9020 B-2015 (5) (j) (7) states: Quality control of commercially prepared media – Test each new lot for sterility and with positive and negative control culture checks. If commercially prepared medium has a longer shelf-life than the laboratory-prepared version, perform these tests more frequently. **Due to the reasons given in question #1, NC WW/GW LC will require a culture positive (no culture negative) <u>once per week for purchased premade media</u> and <u>once per prepared batch for laboratory prepared media</u> in lieu of the above requirements at this time.** NC WW/GW LC Policy: A culture positive must be analyzed with each batch of prepared media and once per week for purchased ready-to-use media
102	Are the culture positive plates countable? [NC WW/GW LC Policy]			A culture positive must be analyzed with each batch of prepared media and once per week for purchased ready-to-use media. A sample volume that yields a countable plate must be analyzed so that individual colonies may be verified to have proper morphology (i.e. color, shape, size, surface appearance). **Often culture positives are TNTC. The analyst must set a volume that yields a countable plate. This does not necessarily mean in the range of 20-60 CFU. The point of a culture positive is beyond just the ability to grow colonies but also to be able to discern individual colonies for proper morphology – that is color, shape, surface appearance, size etc. A sample (e.g., stream samples) may also serve as a culture positive if identified as such.**
103	Are sterility checks (blanks) performed on the entire process at the beginning and end of each filtration series of samples, using 20 to 30 ml of sterile reagent or dilution water as the sample? [SM 9222 D-2015 (2)]			9222 D-2015 (2): Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30mL of dilution or rinse water through filter. 9020 B-2015. (9) (d) (1): For each manifold used in membrane filter tests, check sterility of the entire process by using sterile dilution water as the sample at the beginning and end of each filtration series of samples and test for growth.
104	If there is an interruption of more than 30 minutes in the filtration sequence, are new sterilized funnels used and the sterility test repeated? [SM 9020 B-2015 (9) (d) (1)]			If a processing interruption lasts > 30 min, use new sterilized funnels and repeat sterility test.
105	What corrective action is taken when contamination is apparent? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			Samples results must be qualified.
106	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)]			**At this time, we will follow our Rules for duplicate frequency.** 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.
107	What is the acceptance criterion for duplicates? [15A NCAC 2H .0805 (a) (7)] and [15A NCAC 2H .0805 (a) (7) (A)] **ANSWER:**			Unless specified by the method or this Rule, each laboratory shall establish performance acceptance criteria for all quality control analyses. If the laboratory has different acceptance criteria for plate counts with greater than and less than 20 CFUs, they must establish which acceptance criterion will be

				used to evaluate the duplicates (e.g., plates with 18 and 22 CFUs). Supporting records shall be maintained as evidence that these practices are being effectively carried out. The quality control document shall be available for inspection by the State Laboratory. If an RPD limit between colony counts is used, the mean in the calculation should be an arithmetic mean. If reporting an average of duplicate results(instead of reporting both individual results), the DWR Water Quality Permitting Section has stipulated that it must be the geometric mean; not the arithmetic mean. Keep in mind we are not talking about reporting the duplication of one dilution out of a series of dilutions. Those would be figured into the single result for that sample and not independently reported. This only applies if the entire sample was duplicated or more than one sample was collected in single day.
108	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:**			
109	Is reagent water testing being performed? [NC WW/GW LC 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.
110	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)]			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. If data qualifiers are used to qualify samples not meeting QC requirements, the data may not be useable for the intended purposes. It is the responsibility of the laboratory to provide the client or end-user of the data with sufficient information to determine the usability of the qualified data.

Fecal Coliform-MF is a method-defined parameter.

Additional Comments:

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Inspector: _____Date:_____

The following Fecal Coliform parameter codes have been removed from the allowable parameter list for DMRs: 31613 and 31615.

The only allowable Fecal Coliform parameter code in BIMS is 31616. This is regardless of technology (e.g., Colilert-18, MF, MPN). The parameter descriptor has been modified to "Coliform, Fecal".

The allowable units of measure for 31616 are: 1. Most Probable Number (MPN) per 100mL (MPN/100mL); 2. Number per 100mL (#/100mL); and 3. Colony Forming Units per 100mL (CFU/100mL).