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: **Total Coliform**
Method: **SM 9222 B-2015 (MF) (Aqueous)**
SM 9020, 9030, 9050-2015; SM 9040, 9060-2013

EQUIPMENT: Not all items are required

	Incubator, 35 ± 0.5 ºC (calibrated or accurate to ± 0.2 °C accuracy – see explanation for question #86) **Type of incubator (waterbath or air):**		Bunsen Burner (or flame source) with alcohol to flame
	Thermometer 0.1 °C increments		Dilution bottles
	Sterile sample bottles		Autoclave
	Sterile pipettes		• Temperature gauge
	Graduated cylinders		• Pressure gauge
	Microscope w/ 10-15x magnification		• Holding thermometer
	Sterile metal forceps		Vacuum source
	Sterilizer oven 170 ºC		Sterile non leaking filtration apparatus
	Hotplate w/ magnetic stirrer		Colony counter
	Forceps		Refrigerator

CONSUMABLES: Not all items are required

	Waterproof plastic bag enclosures		Petri dishes
	Sterile absorbent pads		Sterile membrane filters

REAGENTS: Not all items are required

	m-Endo broth		Sterile dilution/ rinse water
	m-Endo agar		• phosphate buffer (KH_2PO_4)
	NaOH		• $MgCl_2$

SM 9020 A-2015 states: QC requirements in section 9020 are not mandatory. Each laboratory develops its own quality management system (QS) suitable for its needs. A laboratory documents its QS's policies and objectives in a quality management plan or quality manual. The document denotes the laboratory's commitment to the QA program for integration of intra- and inter-laboratory QC activities, standardization of laboratory operating procedures, and management practices. It also clearly defines responsibilities and duties to ensure that the data are the type, quality, and quantity required. The program should be practical. Staff should spend about 15% of overall laboratory time on the various aspects of an established QA program. That said, more time may be needed for crucial analytical data (e.g., data for enforcement actions). When properly administered, a balanced, conscientiously applied QS will optimize data quality, identify problems early, and increase satisfaction with analytical results without affecting laboratory productivity.

Based upon this language, in conjunction with method specified requirements, the NC WW/GW LC Branch has established minimum requirements for maintaining certification from our program. These are addressed in this check list along with recommendations to be considered as the laboratory's QC program evolves over time.

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 02H .0805 (a) (7)] **Date:**			Quality assurance, quality control, and Standard Operating Procedure documentation shall indicate the effective date of the document and be reviewed every two years and updated if changes in procedures are made. Verify proper method reference. During review notate deviations from the approved method and SOP.

2	Are all review/revision dates and procedural edits tracked and documented? [15A NCAC 02H .0805 (a) (7)]			Each laboratory shall develop documentation outlining the analytical quality control practices used for the Parameter Methods included in its Certification, including Standard Operating Procedures for each certified Parameter Method. 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. 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.
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)]			Collect samples for microbiological examination in clean, sterile, wide-mouth, nonreactive borosilicate glass or plastic bottles, or in presterilized plastic bags appropriate for microbiological use. The bottles should have non-leaking ground glass stoppers or caps with nontoxic liners that should withstand repeated sterilization. Sterilized as directed in section 9030 B (19) and 9040.
5	Is residual chlorine neutralized at time of sample collection with sterile 0.008% $Na_2S_2O_3$? [40 CFR 136.3 Table II, footnote 5] [SM 9060 A-2013 (2)]			When sampling chlorinated wastewater effluents, add enough $Na_2S_2O_3$ to a clean sample bottle so the final concentration in the sample is 100 mg/L. For example, in a 120-mL bottle, 0.1 mL of a 10% solution of $Na_2S_2O_3$ will neutralize a sample containing up to 15 mg/L residual chlorine.
6	Are samples iced to <10 ºC during shipment? [40 CFR 136.3 Table II]			40 CFR footnote 2 allows 15 minutes for sample preservation, including thermal. This means that if a sample is received in the lab within 15 minutes it is not required to be on ice.
7	Are samples checked for residual chlorine prior to analysis? [40 CFR 136.3 Table II] [NC WW/GW LCB Sample Collection, Preservation and Receipt Requirements Policy]			Each chemically preserved sample must be checked for effectiveness and the results documented. Dechlorinating agents used at the time of sampling must be documented to have been effective (either by the sample collector or the receiving laboratory) by verifying a chlorine residual <0.5 mg/L at a neutral pH.
8	What action is taken if chlorine is present [15A NCAC 02H .0805 (a) (7) (M)]? **Answer:**			If another sample cannot be collected, dechlorinate the sample and notify NC WW/GW Laboratory Certification Branch that a non-compliant sample was received. Reported results must also be qualified.
9	Are samples stored at <10 ºC prior to analysis? [40 CFR 136.3 Table II]			
10	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.**
	MEDIA	**LAB**	**SOP**	**EXPLANATION**
11	If <u>purchased ready-to-use media</u> 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.
	m-Endo Agar			
	Is agar used? If not, skip to question #18.			

12	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (*j*) (1)] [NC WW/GW LCB Traceability Documentation Requirements for Chemicals, Reagents, Standards and Consumables Policy]			**SM 9020 B (5) (j) (1) states:** Document preparation activities such as name of media, volume produced, format, final pH, date prepared, and name of preparer. **Policy states:** A system (e.g., traceable identifiers) must be in place that links standard/reagent preparation information to analytical batches in which the solutions are used. Documentation of solution preparation must include the analyst's initials, date of preparation, the volume or weight of standard(s) used, the solvent and final volume of the solution. This information as well as the vendor and/or manufacturer, lot number, and expiration date must be retained for primary standards, chemicals, reagents, and materials used for a period of five years. Consumable materials such as pH buffers, **lots of pre-made standards and/or media, solids and bacteria filters, etc. are included in this requirement.**
13	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)]			
14	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
15	Is media stirred while heating? [SM 9020 B-2015 (5) (*j*) (1)]			**SM 9020 B (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-magnetic stirrer combinations.
16	Is pH of the m-Endo medium adjusted if necessary and documented to be 7.2 ± 0.2 S.U.? [SM 9222 B-2015 (2) (b) (1)] [SM 9020 B-2015 (5) (j) (1)]			**SM 9222 B (2) (a) states:** Final pH should be 7.2 ± 0.2 S.U. **SM 9020 B (5) (j) (1) 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 1N 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.)
17	Is agar media stored in refrigerator and discarded after 2 weeks? [SM 9222 B-2015 (2) (b) (1)] Skip to question #25.			**SM states:** Refrigerate finished medium in the dark and discard unused agar after 2 weeks [or sooner if there is evidence of moisture loss, medium contamination, medium deterioration (darkening of medium), or surface sheen formation].
	m-Endo Broth			
18	Is the m-Endo media purchased pre-made or prepared in the lab? If purchased premade skip to question #25.			Prepared media includes purchased premixed media that is rehydrated in the lab.
19	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (*j*) (1)] [NC WW/GW LCB Traceability Documentation Requirements for Chemicals, Reagents, Standards and Consumables Policy]			**SM 9020 B (5) (j) (1) states:** Document preparation activities such as name of media, volume produced, format, final pH, date prepared, and name of preparer.
20	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)]			
21	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
22	Is media stirred while heating? [SM 9020 B-2015 (5) (*j*) (1)]			**SM 9020 B (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-magnetic stirrer combinations.
23	Is pH of the m-Endo medium adjusted if necessary and documented to be 7.2 ± 0.2 S.U.? [SM 9222 B-2015 (2) (*b*) (2)] [SM 9020 B-2015 (5) (j) (1)]			**SM 9222 (2) (b) (2) states:** Prepare as above, omitting agar (i.e., Final pH should be 7.2 ± 0.2 S.U.). **SM 9020 B (5) (j) (1) 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 1N 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.)
24	Is broth stored in refrigerator and discarded after 96 hours? [SM 9222 B-2015 (2) (*b*) (2)]			
	VERIFICATION MEDIA	**LAB**	**SOP**	**EXPLANATION**
25	Is the LTB medium purchased ready-to-use or prepared in the lab? If purchased ready-to-use skip to question #46.			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.
26	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			See explanation in question 19.
27	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)]			
28	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
29	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.
30	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.
31	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.
32	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
33	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.
34	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.
35	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

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				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).
36	Is the BGB medium purchased ready-to-use or prepared in the lab? If purchased ready-to-use skip to question #46.			Although SM 9221 B-2014 (4) (*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.
37	If prepared in the lab, is the preparation documented? [SM 9020 B-2015 (5) (j) (1)]			
38	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)]			
39	Is reagent grade water used in preparing media? [SM 9020 B-2015 (5) (j) (1)]			
40	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.
41	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 (4) (*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.
42	Is medium autoclaved at 121°C for 12 to 15 minutes in capped tubes? [SM 9221 B-2014 (4) (*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.
43	After sterilization, are inverted vials free of air bubbles? [SM 9221 B-2014 (4) (*a*)]			Ensure that inverted vials are free of air bubbles.
44	Is pH of the BGB medium adjusted if necessary and documented to be 7.2 ± 0.2 S.U.? [SM 9221 B-2014 (4) (*a*)] and [SM 9020 B-2015 (5) (j) (1)]			SM 9221 B-2014 (4) (*a*) states: Medium pH should be 7.2 ± 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 7.2 ± 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).
45	When prepared in-house, are the BGB and LTB 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.
	PROCEDURE	**LAB**	**SOP**	**EXPLANATION**
46	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 SM 9020 B-2015, Table: IV. Final pH **should** be 7.2 ± 0.1 S.U. Note that pH values will change with time. Store under refrigerated conditions after opening and discard if turbidity develops. Use within 6 months.

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				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 1*N* 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. 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 growth (in peptone water) may occur.
47	Are sample volumes selected to yield counts between 20 and 80 colonies per membrane? [SM 9222 B-2015 (4) (a)]			An ideal sample volume will yield 20 to 80 total coliform colonies and ≤200 colonies of all types (typical, atypical, and non-coliform background colonies) on a membrane-filter surface (Table 9222: II).
48	Are at least three dilutions analyzed? [SM 9222 B-2015 (4) (a)]			Analyze other waters by filtering three different volumes (diluted or undiluted), depending on the expected bacterial density.
49	How are samples homogenized prior to filtration? [SM 9222 B-2015 (4) (c)] [SM 9060 A-2013 (3)] **Answer:**			**SM 9222 B (4) states:** 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. **SM 9060 A (3) states:** Bottles used for sample collection should be large enough to collect desired volume and still maintain adequate headspace (2.5 cm) to ensure proper sample mixing (via shaking) before analyses. If a sample bottle arrives at the laboratory without adequate headspace for proper mixing, either reject it and request resampling or else (to maintain sample integrity) pour entire sample volume into a sterile container large enough to ensure adequate mixing and then withdraw 100 mL (or required sample volume) aseptically into another suitably sized sterile container.
50	When less than 10 mL of sample is to be filtered, is 10 mL of sterile buffered dilution water, then sample, and an additional 25-50 mL of dilution water added to the funnel before filtration begins? [SM 9222 B-2015 (4) (a)]			(See Section 9215B.2 for preparation of dilutions.) When filtering <10 mL of sample (diluted or undiluted), add approximately 10 mL sterile buffered dilution water to the funnel and then add sample followed by another 25 to 50 mL dilution water before filtration or pipet the sample volume into sterile dilution water and then filter the entire contents of dilution bottle. This increase in water volume helps disperse the bacterial suspension uniformly over the entire effective filtering surface.
51	Are sterile forceps used to place the sterile membrane filter (grid side up) on the filter plate? [SM 9222 B-2015 (4) (c)]			Forceps are sterilized by alcohol flaming.
52	If 30 minutes or longer elapses between sample filtrations, are all common apparatuses that come in contact with samples re-sterilized? [SM 9222 B-2015 (4) (b)]			Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is interrupted when an interval of 30 min or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use. (See 9222B.1*f* for sterilization procedures and Sections 9020B.4*l* and *m* for UV cleaning and safety guidelines.)
53	Is the sample filtered under partial vacuum? [SM 9222 B-2015 (4) (c)]			Commonly used pressure: 81 kPa, 24 in. Hg, or 79% vacuum.
54	With the filter still in place, is the interior surface of the filter funnel rinsed with three 20-30mL portions of sterile dilution water? [SM 9222 B-2015 (4) (c)]			Use 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.
55	Are absorbent pads used in conjunction with m-Endo broth medium? [SM 9222 B-2015 (2) (b) (2)]			Dispense liquid medium (at least 2.0 mL per plate) onto sterile absorbent pads (see 9222B.1h) and carefully remove excess medium by decanting plate. The broth may have a precipitate, but this does not interfere with medium performance if pads are certified free of sulfite or other toxic agents at concentrations that could inhibit bacterial growth.
56	While in the culture dish, is the pad saturated with at least 2.0 to 3.0 mL of m-Endo medium and the excess decanted from the dish? [SM 9222 B-2015 (4) (*c*)]			
57	Are prepared filters aseptically placed directly on the selected medium with a rolling motion to avoid entrapment of air? [SM 9222 B-2015 (4) (*c*)]			
58	How are prepared dishes placed in the incubator? [SM 9222 B-2015 (1) (*j*) and (4) (*c*)] **Answer:**			Place all prepared dishes in a sealed container with tight-fitting lid (or sealed bag), invert and place in a waterbath or humid chamber (or humidified incubator). **SM 9222 B (1) (j) states:** To avoid excessive drying, maintain a humid environment for the plates during incubation by either using a humidified incubator (between 60 and 90% relative humidity) or placing plates in a seal container with tight-fitting lid (or sealed bag). **SM 9222 B (4) (c) states:** [Agar] Invert dish and incubate at 35 ± 0.5 °C for 22 to 24 h. [Broth] Place sample filter directly on pad, invert dish, and incubate as specified above. If loose-lidded dishes are used, place them in a humid chamber (or humidified incubator)
59	Are the date and time samples are placed in the incubator documented? [15A NCAC 02H .0805 (a) (7) (F)]			The date and time that samples are placed into and removed from ovens, water baths, incubators and other equipment shall be documented if a time limit is required by the method.
60	Are samples incubated at 35 ± 0.5 ºC? [SM 9222 B-2015 (4) (*c*)]			Invert dish and incubate at 35 ± 0.5 °C for 22 to 24 h for m-Endo LES or m-Endo MF.
61	Are samples incubated for 22 - 24 hours? [SM 9222 B-2015 (4) (*c*)]			Invert dish and incubate at 35 ± 0.5 °C for 22 to 24 h for m-Endo LES or m-Endo MF.
62	Are the date and time samples are removed from the incubator documented? [15A NCAC 02H .0805 (a) (7) (F)]			The date and time that samples are placed into and removed from ovens, water baths, incubators and other equipment shall be documented if a time limit is required by the method.
63	Are both typical and atypical colonies counted? [SM 9222 B-2015 (4) (*f*)]			The angle of light on the colony affects sheen detection for coliform colonies growing on m-Endo plates. Rocking and turning the Petri plate reflects light at different angles and helps detect sheen on the colony. The typical coliform colony on Endo-type media has a pink to dark-red color with a metallic surface sheen. Count both typical and atypical coliform colonies promptly after incubation. The sheen area may vary in size from a small pinhead to complete coverage of the colony surface. Atypical coliform colonies can be dark red, mucoid, or nucleated without sheen. Generally pink, blue, white, or colorless colonies lacking sheen are considered non-coliforms.
64	What type of optical device is used for counting colonies? [SM 9222 B-2015 (4) (*f*)] **Answer:**			To count colony-forming units (CFU) on Endotype membrane filters, use a low-power (10 to 15x magnification) binocular wide-field dissecting microscope or other optical device, with a cool white fluorescent light source directed to provide optimal viewing of sheen.
65	Is the density of CFU calculated according to the method? [SM 9222 B-2015 (5)]			CFU/100 mL = $\frac{\text{coliform colonies counted x 100}}{\text{mL sample filtered}}$
66	Are results reported according to the NC WW/GW LCB Fecal Coliform Reporting policy document?			
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
67	Are filtration units sterilized via autoclave prior to the beginning of each filtration series? [SM 9222 B-2015 (4) (*b*)]			Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. **A filtration series is interrupted when an**

<table>
<tr><td></td><td></td><td></td><td></td><td>interval of 30 min or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use. (See 9222B.1f for sterilization procedures and Sections 9020B.4l and m for UV cleaning and safety guidelines.)</td></tr>
<tr><td>68</td><td>Is the autoclave capable of reaching at least 121ºC within 15 minutes? [SM 9030 B-2015 (3)]</td><td></td><td></td><td>Use autoclaves large enough to prevent internal crowding; constructed to provide uniform temperatures within the chambers (up to and including the sterilizing temperature of 121 C); and capable of reaching the desired temperature within 15 min.</td></tr>
<tr><td>69</td><td>Is an autoclave log maintained that documents all required information? [15A NCAC 02H .0805 (a) (7) (I)]</td><td></td><td></td><td>During each use of an autoclave, the temperature, pressure, cycle time, and items autoclaved shall be checked, recorded, dated, and initialed.</td></tr>
<tr><td>70</td><td>Are all applicable consumables and media sterilized at 121°C? [SM 9020 B-2015 (5) (j) (2)] [SM 9050 A-2015 (A) (3)]</td><td></td><td></td><td>TABLE 9020: IV: TIME AND TEMPERATURE FOR AUTOCLAVE STERILIZATION*
<table>
<tr><th>Material</th><th>Time at 121 °C min</th></tr>
<tr><td>Membrane filters and pads</td><td>10</td></tr>
<tr><td>Carbohydrate-containing media (lauryl tryptose, BGB broth, etc.)</td><td>12-15†</td></tr>
<tr><td>Contaminated materials and discarded cultures</td><td>30</td></tr>
<tr><td>Membrane filter assemblies (wrapped), sample collection bottles (empty)</td><td>15</td></tr>
<tr><td>Buffered dilution water, 99 mL in screw-cap bottle</td><td>15</td></tr>
<tr><td>Rinse water, volume >100 mL</td><td>Adjust for volume</td></tr>
</table>
* Except for media, times are guidelines
† Certain media may require different sterilization conditions</td></tr>
<tr><td>71</td><td>Is media removed from the autoclave within the proper timeline? [SM 9050 A-2015 (A) (3)]</td><td></td><td></td><td>Sterilize media in an autoclave at 121 ºC. Review method and manufacturer’s requirements. The required exposure time will vary with form and type of material, medium, presence of carbohydrates, and volume. Sterilize most carbohydrate broths at 121 ºC for 12 to 15 min; however, there are exceptions. For example, A-1 media must be autoclaved for 10 min at 121 ºC. When pressure reaches zero, remove medium from autoclave and cool quickly to avoid decomposition of sugars due to prolonged heat exposure. To permit uniform heating and rapid cooling, loosely pack materials into small containers. The maximum heat exposure for most carbohydrate broths (from closing loaded autoclave to unloading) is <45 min. The maximum heat exposure or A-1 medium is <30 min. Preferably use a double walled autoclave to permit preheating before loading to keep total heating time within the limit. Adjust autoclave times as volumes/loads increase. Presterilized media may be available commercially. Do not re-autoclave media.</td></tr>
<tr><td>72</td><td>Is heat indicating tape used with all materials each sterilizing cycle? [SM 9020 B-2015 (4) (h)]</td><td></td><td></td><td>Not required but recommended. This QC section now simply states that heat-indicating tape can quickly identify supplies and materials that have been sterilized.</td></tr>
<tr><td>73</td><td>Is the autoclave temperature checked weekly with a maximum registering thermometer or HTDL and documented? [SM 9020 B-2015 Table 9020: I] [SM 9020 B-2015 (4) (h)]</td><td></td><td></td><td>For routine use, verify autoclave temperature weekly with a maximum registering thermometer (MRT) (generally a mercury-filled Teflon-coated device) or accurate HTDL (high-temperature data logger), able to withstand 15 to 20 lb/in. sq. If neither device is available, use a strip or pie chart recorder with interpretations written on the chart.

Annual calibration is not required.</td></tr>
</table>

74	Is glassware not in metal containers sterilized at ≥ 170ºC oven for at least 2 hours or autoclaved at 121°C for at least 30 min? [SM 9040-2013]			To sterilize glassware via dry heat, use a hot-air oven set at ≥170°C for 2 h or longer. Alternatively, sterilize glassware by autoclaving at 121°C for at least 30 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. Note: The term glassware refers to both borosilicate glass and heat-resistant plastic materials.
75	Is glassware in metal containers sterilized at ≥ 170ºC oven for at least 2 hours? [SM 9040-2013]			For glass pipets in metal containers, sterilize using a hot-air oven set at ≥170°C for at least 2 h.
76	How are sample bottles sterilized? [SM 9040-2013] **Answer:**			To sterilize glassware via dry heat, use a hot-air oven set at ≥170°C for 2 h or longer. Alternatively, sterilize glassware by autoclaving at 121°C for at least 30 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.
77	Is the laboratory purchasing pre-sterilized sample vessels or sterilizing reusable bottles? **Answer:**			
78	Are laboratory sterilized bottles checked for sterility? [SM 9020 B-2015 (5) (*d*)] [NC WW/GW LCB Bacteriological Sample Bottle Sterility Test Policy]			**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. Document results. If growth occurs, discard entire batch or lot. We will accept Certificate of Analysis for store bought bottles or sample bags in lieu of the above testing.
79	Are Ultra-Violet (UV) lamps used to sanitize the filtering apparatus between samples? [SM 9222 B-2015 (4) (*c*)] If not, skip to question 83.			Optionally, 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.
80	Are UV lamp bulbs cleaned monthly with a soft cloth moistened with ethanol? [SM 9020 B-2015 (4) (*l*)]			Ultraviolet lamps: Disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol.
81	Are UV lamp bulbs tested quarterly with an appropriate UV light meter? [SM 9020 B-2015 (4) (*l*)]			Test lamps quarterly with an appropriate UV light meter.
82	Are UV lamp bulbs replaced if the output is less than 70% of the original? [SM 9020 B-2015 (4) (*l*)]			Replace bulbs if the output is less than 70% of the original.
83	Is the incubator temperature monitored and documented twice daily separated by 4 hours? [SM 9020 B-2015 (4) (*n*) and (*o*)]			Verify that incubators maintain the set temperature. When incubator is in use (i.e., samples are being incubated), monitor and record corrected twice daily separated by 4 h.
84	Is the thermometer/temperature measuring device graduated in 0.1°C increments? [SM 9030 B-2015 (12)]			Thermometers must be graduated in appropriate increments; for example, use thermometers graduated to 0.1 °C for incubators operated >35 °C (Note for auditor) 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.
85	Is accuracy of the thermometer/ temperature measuring device verified against a Reference Temperature-Measuring Device every three months? [15A NCAC 02H .0805 (a) (7) (N) (iii)] [NC WW/GW LCB Temperature-Measuring Devices used for Laboratory Operations Policy]			**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. **Policy:** Incubators with an incubation temperature tolerance of ± 0.5 °C (e.g., total coliform and *E. coli* incubators) must have temperature measuring devices with a stated accuracy of at least ± 0.2 °C. These devices must be able to distinguish temperature changes of 0.1°C and equilibrate rapidly. 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.

86	Is the temperature correction posted? [SM 9020 B-2015 (4) (*a*)]			If a correction calculation is necessary, mark the appropriate correction factor on the device so only corrected temperature values are recorded.
87	Is a culture positive analyzed with each batch of prepared media? [SM 9222 B-2015 (2)]			Before use, test each batch of laboratory-prepared MF medium for performance with positive and negative culture controls.
88	Does the culture positive plate show individual colonies with proper morphology? [NC WW/GW LCB Coliform Membrane Filter Culture Positive Policy]			A culture positive must be analyzed with each batch of prepared media and once per week for purchased ready-to-use media. The sample volume used must yield a plate showing individual colonies with proper morphology (e.g., color, shape, size, surface appearance). 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.
89	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 02H .0805 (a) (7) (C)]			**Rules:** 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 (i.e., 5% or 1 per month when <20 samples are analyzed per month).**
90	What is the acceptance criterion for duplicates? [15A NCAC 02H .0805 (a) (7) and .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.
91	What corrective action does the laboratory take if the duplicate sample results are outside of established control limits or method precision limits? [15A NCAC 02H .0805 (a) (7) (B)] **Answer:**			
92	Are sterility checks (blanks) performed on the entire process at the beginning and end of each filtration series of samples, using sterile reagent or dilution water as the sample? [SM 9222 B-2015 (4) (*d*)]			Check for sterility and coliform contamination at the beginning and end of each filtration series, respectively, by filtering 20 to 30 mL of dilution or rinse water through the filter (one funnel per sterilization batch).

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93	Is a 100 mL blank analyzed after every 10th filtration? [SM 9222 B-2015 (4) (*d*)]			Additionally, to check for possible cross-contamination or contaminated rinse water, insert a sterile rinse-water sample (100 mL) after filtration of 10 samples. Incubate these QC samples under the same conditions as the samples being analyzed.
94	What corrective action does the laboratory take if countable colonies appear in the any of the blanks? [SM 9222 B-2015 (4) (*d*)] **Answer:**			If controls indicate contamination, reject all data from affected samples and request new samples. Qualification is also acceptable if new samples cannot be obtained.
95	On a monthly basis, are at least ten sheen colonies from positive samples verified using lauryl tryptose broth and brilliant green lactose bile broth, followed by count adjustments based on these results? [40 CFR 136.3 Table IB, Footnote 30]			On a monthly basis, at least ten sheen colonies from positive samples must be verified using lauryl tryptose broth and brilliant green lactose bile broth, followed by count adjustment based on these results; and representative non-sheen colonies should be verified using lauryl tryptose broth. Where possible, verifications should be done from randomized sample sources. See Total Coliform (Membrane Filter) Colony Verification Technical Assistance document for guidance.
96	On a monthly basis, are at least ten representative non-sheen colonies verified using lauryl tryptose broth? [40 CFR 136.3 Table IB, Footnote 30]			Recommended, see above.
97	Is reagent water testing performed at least every 12 months? [SM 9020 B-2015 Table 9020:II] [NC WW/GW LCB Bacteriological Reagent Water Testing Policy]			At this time, it is required to test the reagent water for conductivity, TOC, Cd, Cr, Cu, Ni ,Pb, and Zn – reasons for less frequency at this time see question #1. 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.
98	Are results qualified to indicate quality control failures or sample anomalies when reporting results? [15A NCAC 02H .0805 (e) (5)]			Reported data associated with quality control failures, improper sample collection, holding time exceedances, or improper preservation shall be qualified as such.

Additional Comments:

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Inspector: _____ Date: _____