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

Parameter: **Fecal Coliform**
Method: **IDEXX Colilert®-18**

Equipment and Reagents:

	Waterbath Incubator capable of maintaining 44.5 ± 0.2°C Air Incubators are not allowed per 40 CFR 136.3.		Quanti-Trays®: Specify type used. □ Quanti-Tray® □ Quanti-Tray®/2000		Sterile, non-buffered, oxidant-free dilution water
	Quanti-Tray® Sealer				Anti-foam solution
	Quanti-Tray® rubber insert		Sterile inoculating loop		*E. coli*-strain (optional):
	Comparator		Most Probable Number (MPN) Chart		*Pseudomonas aeruginosa*-strain (optional):
	120 ml sterile vessels with $Na_2S_2O_3$		Colilert®-18 reagent		

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 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.
4	Is Colilert®-18 used for fecal coliform analysis on effluent samples only?			Colilert®-18 is not to be used on marine waters per the method. Colilert-18® may <u>not</u> be used for fecal coliform analysis of storm water samples, stream monitoring tied to NPDES permits (e.g., upstream, downstream), groundwater monitoring wells or for biosolids monitoring under 503 regulations. It may; however, be used for any wastewater analysis including when wastewater is used in an application such as spray irrigation or used for beneficial reuse as reclaimed water.
	PRESERVATION and STORAGE	**LAB**	**SOP**	**EXPLANATION**
5	Are samples collected in sterile containers using aseptic technique? [Colilert®-18, Procedural Notes]			Aseptic technique should always be followed when using Colilert-18.
6	Is residual chlorine neutralized at time of sample collection with sterile $Na_2S_2O_3$? [40 CFR 136.3 Table II, Footnote 5] [SM 9060 A-2013 (2)]			**SM 9060 A-2013:** When sampling chlorinated wastewater effluents, add enough $Na_2S_2O_3$ 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.
7	Are samples iced to above freezing but <10 ºC during transport? [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.
8	Are samples checked for residual chlorine prior to analysis in the laboratory? [40 CFR 136.3 Table II] [15A NCAC 02H .0805 (a) (7) (M)]			Use of TRC strips is allowed, see NC WW/GW LCB Sample Collection, Preservation and Receipt Requirements Policy.
9	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 the NC WW/GW Laboratory Certification Branch that a non-compliant sample was received and analyzed. The sample must be qualified with the nature of the infraction on the DMR and/or client report.

10	Are samples placed into the incubator within 8 hours of collection? [40 CFR 136.3 Table II]			Sample analysis should begin as soon as possible after receipt; sample incubation must be started no later than 8 hours from time of collection.
11	Are samples stored at <10 °C prior to analysis? [40 CFR 136.3 Table II]			
	PROCEDURE – Sample Preparation	**LAB**	**SOP**	**EXPLANATION**
12	Is Colilert-18 reagent stored at 2-25°C away from light? [IDEXX Colilert-18 Method, Storage]			Store at 2-25°C away from light.
13	Are samples brought to room temperature prior to analysis? [IDEXX Colilert-18 Method, Quanti-Tray Enumeration Procedure]			Add contents of one pack to a 100-mL room temperature water sample in a sterile vessel.
14	Is the content of one pack added to a 100-mL sample in a sterile vessel? [IDEXX Colilert-18 Method, Quanti-Tray Enumeration Procedure]			Add contents of one pack to a 100-mL room temperature water sample in a sterile vessel. A slight tinge may be observed when Colilert-18 is added to the sample. In samples with excessive chlorine, a blue flash may be seen when adding Colilert-18.
15	Is the sample shaken until the contents are dissolved? [IDEXX Colilert-18, Quanti-Tray Enumeration Procedure]			Cap vessel and shake until dissolved.
16	If foaming occurs after the addition of Colilert-18 reagent, what action is taken? [EPA Region 4 (Science and Ecosystem Support Division) Approval of Colilert-18 for the Detection and Enumeration of Fecal Coliforms in Wastewater Samples Policy, Section 2 (C).] [Quanti-Tray Enumeration Procedure] **Answer:**			**EPA Region 4 (Science and Ecosystem Support Division) Approval of Colilert-18 for the Detection and Enumeration of Fecal Coliforms in Wastewater Samples Policy**: If foaming occurs, after the addition of Colilert-18 to the sample, then add Antifoam solution (IDEXX part #WAFDB) to sample. Alternatively, allow the sample to sit for several minutes prior to adding it to a Quanti-Tray. **Colilert-18 Method**: If excess foam caused problems while using Quanti-Tray, you may choose to use IDEXX Antifoam Solution (Catalog# WAFDB) OR IDEXX 120 ml vessels with Antifoam (Catalog# WV120SBAF-200).
17	Are sealed trays incubated at 44.5°C ± 0.2°C? [IDEXX Colilert-18 Method]			Place the sealed tray in a … (44.5 ± 0.2°C for fecal coliforms) incubator for 18 hours. Pre-warming to 35°C is not required per the method and the ***Colilert-18 Fecal Coliform Protocol Addendum***.
18	Are samples incubated for 18-22 hours? [IDEXX Colilert-18 Method]			Place the sealed tray in a 44.5 ± 0.2°C for fecal coliforms incubator for 18 hours. **EPA Region 4 (Science and Ecosystem Support Division) Approval of Colilert-18 for the Detection and Enumeration of Fecal Coliforms in Wastewater Samples Policy**: Place sealed tray in a 44.5 ± 0.2°C incubator for 18-22 hours. The usual pre-warming to 35°C is not required since the sample is first placed in the Quanti-tray sealer prior to incubation. (Note: Temperature was increased from 35°C to 44.5°C to facilitate fecal coliform growth rather than total coliform.)
19	Is the time that 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
20	Is the incubator temperature documented? [15A NCAC 02H .0805 (a) (7) (I)]			Each day samples are placed into or removed from an incubator, oven, water bath, refrigerator, or other temperature-controlled device, the temperature shall be checked, recorded, dated, and initialed.
21	Is the time that 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
22	Is sterile, non-buffered, oxidant-free dilution water used to make dilutions when required? [IDEXX Colilert-18, Procedure Notes]			Use only sterile, non-buffered, oxidant-free water for dilutions.
23	Is a water bath incubator used? [40 CFR 136.3 Table IA, Footnote 28]			To use Colilert-18® to assay for fecal coliforms, the incubation temperature 44.5 ± 0.2 °C, and a water bath incubator is used.
24	Are trays fully immersed and weighted in the water bath (i.e., not in plastic bags)? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure]			For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.

	PROCEDURE – Sample Analysis	LAB	SOP	EXPLANATION
25	Is a comparator used to determine color intensity for result interpretation? [IDEXX Colilert®-18, Result Interpretation]			**Colilert®-18 Fecal Coliform Protocol Addendum:** IDEXX P/A Comparator catalog# WP104, Quanti-Tray Comparator# WQTC or Quanti-Tray/2000 Comparator# WQT2KC. Yellow equal to or greater than the comparator when incubated at 44.5±0.2°C: Positive for fecal coliforms.
26	Are the number of positive wells counted and recorded for large and small wells on a laboratory benchsheet? [IDEXX Colilert®-18 Method] [15A NCAC 02H .0805 (a) (7) (F) (xviii)]			**Rule:** All laboratories shall use printable laboratory benchsheets. Certified Data shall be traceable to the associated sample analyses and shall consist of: any other data needed to reconstruct the final calculated result. **IDEXX Colilert-18 Method**: Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.
27	Is the MPN Table provided with the trays used to obtain a Most Probable Number? [IDEXX Colilert®-18 Method]			Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.
28	Are results multiplied by an appropriate dilution factor when sample dilutions are made? [06-31855-00 IDEXX]			[06-31855-00: Diluting water samples for use with the Quanti-Tray or Quanti-Tray/2000 system] To correct the result for dilution, multiply the MPN value by the dilution factor.
29	If multiple dilutions of the same sample are analyzed, is the result reported from the least diluted sample that provides a readable tray? [06-31855-00 IDEXX]			Use the least-diluted sample that provides a readable tray with results that most closely approach 70%-80% positive wells. Sensitivity may be lost with each increasing dilution. **IMPORTANT:** Never average the MPN values from different dilutions.
30	How is the sample result reported when analyzed in duplicate? **Answer:**			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. This also applies if more than one sample is analyzed in a day.
	QUALITY ASSURANCE	**LAB**	**SOP**	**EXPLANATION**
31	Are laboratory sterilized bottles used for sample collection checked for sterility? [NC WW/GW LCB Bacteriological Sample Bottle Sterility Test Policy]			Minimally test for sterility one sample bottle per batch sterilized in the laboratory, or at a set percentage such as 1 to 4%. This is performed by adding sterile dilution/rinse water to the bottle after sterilization and then subsequently analyzing it as a sample. Document results. If sample bottles or bags are purchased pre-sterilized, verification of sterilization is not required if the laboratory maintains copies of the Certificate of Analysis from the vendor.
32	[Optional] Is each lot of Colilert®-18 checked with one fecal coliform strain and one non-fecal coliform strain? [**Recommended** in Colilert®-18 Fecal Coliform Protocol Addendum]			**Recommended**. The **Colilert®-18 Fecal Coliform Protocol Addendum** states: The following quality control procedure is **recommended** for each lot of Colilert-18 when used for fecal coliform testing at 44.5°C ± 0.2°C: 1. Fill two sterile vessels with 100 ml sterile water and inoculate one with a fecal coliform (*E. coli*) and one with a non-fecal coliform (*Pseudomonas aeruginosa*) using the following recommended strains: A sterile loop of American Type Culture Collection (ATTC) strains, *E. coli* ATCC25922 or 11775 and *Pseudomonas aeruginosa* ATCC 10145 or 27853. 2. Follow the Quanti-Tray Enumeration Procedure above. 3. Results should match the Result Interpretation table above.

33	What corrective action is taken if the results do not match the Result Interpretation table? [15A NCAC 02H .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.
34	If the original sample has some background color, is the inoculated Colilert-18 sample compared to a control blank of the same water sample to confirm yellow color change is greater than background color of sample? [IDEXX Colilert®-18 Method, Procedural Notes]			If a water sample has some background color, compare inoculated Colilert®-18 sample to a control blank of the same water sample.
35	Is the sealer checked monthly? [NC WW/GW LCB Quanti-Tray® Sealer Check Policy]			If the Quanti-Tray® or Quanti-Tray®/2000 test is used, the sealer must be checked monthly by adding a dye (e.g., food color or bromocresol purple to a water blank.
36	What corrective action is taken if the seal is not adequate? [NC WW/GW LCB Quanti-Tray® Sealer Check Policy] **Answer:**			If dye is observed outside the wells, either perform maintenance or use another sealer.
37	Is reagent water testing being performed? [NC WW/GW LCB Bacteriological Reagent Water Testing 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.
38	Does the laboratory analyze duplicate samples at a rate of 5%? [15A NCAC 02H .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.
39	What is the acceptance criterion for duplicates? [15A NCAC 02H .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. Each laboratory shall calculate and document the precision and accuracy of all quality control analyses with each sample set. The lab must set an acceptance criterion at all concentration levels. IDEXX recommends basing acceptance on the 95% confidence range. Looking at the sample and duplicate ranges, they are acceptable as long as those 2 ranges overlap. Go to the following website to download a program where you can enter results and it will calculate the MPN and 95% confidence range- https://www.idexx.com/en/water/resources/mpn-generator/. Alternately, a chart that contains all possible MPN results with the corresponding 95% confidence levels can be found on the technical assistance portion of our website. RPD may also be used for the acceptance criterion. When calculating the RPD, use the arithmetic mean.

40	What corrective action does the laboratory take if the duplicate sample results are outside of established control limits? [15A NCAC 02H .0805 (a) (7) (C) and (B)] **Answer:**			
41	Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [15A NCAC 02H .0805 (a) (7) (B)]			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.

Additional Comments:

Inspector: _____ Date: _____

Result Interpretation:

Appearance	Result
Less yellow than comparator when incubated at 44.5°C ± 0.2°C.	Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 44.5°C ± 0.2°C.	Positive for fecal coliforms

Colilert-18 results are definitive at 18–22 hours. In addition, positives for fecal coliforms observed before 18 hours and negatives observed after 22 hours are also valid.

Colilert®-18 Fecal Coliform Protocol Addendum

Follow the procedures in this protocol addendum when using Colilert-18 to test for fecal coliforms in wastewater. Please refer to the printed package insert for kit storage requirements or when using Colilert-18 to test for total coliforms/*E. coli*.

Quanti-Tray® Enumeration Procedure

1. Add contents of one snap pack to a 100 mL room temperature water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 44.5˚C ±0.2˚C incubator for 18 hours (prewarming to 35˚C is not required). For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the Quanti-Tray to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator[1] when incubated at 44.5°C ±0.2°C	Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 44.5°C ±0.2°C	Positive for fecal coliforms

- Colilert-18 results are definitive at 18–22 hours. In addition, positives for fecal coliforms observed before 18 hours and negatives observed after 22 hours are also valid.

Quality Control Procedures

The following quality control procedure is recommended for each lot of Colilert-18 when used for fecal coliform testing at 44.5˚C ±0.2˚C:

1. Fill two sterile vessels with 100 mL sterile water and inoculate one with a fecal coliform (*E. coli*) and one with a non-fecal coliform (*Pseudomonas aeruginosa*) using the following recommended strains:
   - A sterile loop of ATTC[2] strains, *E. coli* ATCC 25922 or 11775 and *Pseudomonas aeruginosa* ATCC 10145 or 27853
2. Follow the Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

For IDEXX Technical Support, please call:

North/South America: 207-556-4496/1-800-321-0207

1. IDEXX P/A Comparator, catalog # WP104, Quanti-Tray Comparator # WQTC, or Quanti-Tray/2000 Comparator # WQT2KC
2. American Type Culture Collection 1-800-638-6597

One IDEXX Drive
Westbrook, Maine 04092 USA
www.idexx.com/water
Tel: 1-207-556-4496 or 1-800-321-0207
Fax: 1-207-556-4630

Quanti-Tray* and Quanti-Tray*/2000

English

Diluting water samples for use with the Quanti-Tray* or Quanti-Tray*/2000 system

The maximum counting ranges for Quanti-Tray and Quanti-Tray/2000 greatly reduce the need for manual dilutions. However, if you suspect a sample may be heavily contaminated, follow these guidelines to dilute the sample before testing:

- Dilute the sample if the expected MPN value, based on the MPN table included in the Quanti-Tray insert, will be:
  - >200 per 100-mL sample for the 51-well Quanti-Tray
  - >2,419 per 100-mL sample for the 97-well Quanti-Tray/2000
- Dilute the sample before adding the Colilert*, Colilert*-18, Colisure*, Pseudalert*, Enterolert*, Enterolert*-DW or HPC for Quanti-Tray* reagent to the sample.
- Always use sterile deionized (DI) water as the diluent, as described in the package insert.

Recommended dilutions

Dilution	Sample		Diluent
1:1	50 mL	+	50 mL
1:4	25 mL	+	75 mL
1:5	20 mL	+	80 mL
1:10	10 mL	+	90 mL
1:20	5 mL	+	95 mL

Example using a 1:20 dilution

1. Mix the sample thoroughly and then withdraw 5 mL using a sterile pipette, or equivalent, and transfer sample to a sterile vessel.
2. Add 95 mL of sterile DI water to the vessel.
   Note: Diluent can be added to the vessel first, followed by the sample.
3. Add a packet of reagent to the diluted sample and mix well to dissolve.
4. Pour sample into a Quanti-Tray or Quanti-Tray/2000 tray, seal, and then incubate following instructions in the package insert.
5. Follow instructions in the package insert to record the number of positive wells.
6. Use the appropriate MPN chart (for Quanti-Tray or Quanti-Tray/2000) to obtain the MPN/100 mL value.
7. To correct the result for dilution, multiply the MPN value by the dilution factor (20).

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Correcting the MPN and confidence interval

When a sample has been diluted, you must correct the MPN value and the lower and upper confidence limits (CLs). Multiply these values by the dilution factor.

Example using a 1:20 dilution

Original MPN	64/100 mL
Original 95% CLs	46.8 (lower); 85.1 (upper)
Dilution factor	20 (100 mL / 5 mL)
Corrected MPN	64/100 mL x 20 = 1,280/100 mL
Corrected lower CL	46.8 x 20 = 936
Corrected upper CL	85.1 x 20 = 1,702

Which dilution should be used when reporting Quanti-Tray results?

Use the least-diluted sample that provides a readable tray with results that most closely approach 70%-80% positive wells. Sensitivity may be lost with each increasing dilution.

IMPORTANT: Never average the MPN values from different dilutions.

Example using three dilutions

For this example, three dilutions (1:1, 1:10, and 1:100) are tested using Quanti-Tray/2000. After incubation, the three trays provide the following positive-well counts:

Dilution	Large wells	Small wells
1:1	38	12
1:10	20	7
1:100	8	0

The results from the **1:1 dilution** should be reported, because it is the least-diluted sample that has results closest to 70%–80% positive wells.

The 1:10 dilution would yield a higher MPN compared to the 1:1 dilution, because of the dilution factor of 10. The 1:100 dilution has a large multiplier that would result in an erroneously high number because of overdilution of the sample.

Dilutions may not be necessary when using Quanti-Tray

According to *Standard Methods for the Examination of Water and Wastewater*,[1] section 9222, three dilutions are recommended for membrane filtration in order to yield 20–80 colonies/plate for total coliforms and *E. coli,* and 20–60 colonies/plate for fecal coliforms.

However, when using Quanti-Tray or Quanti-Tray/2000, two to three dilutions are usually not needed. For a 100-mL sample, Quanti-Tray has a most-probable-number (MPN) range of up to 200 and Quanti-Tray/2000 has a range of up to 2,419. Even when samples are heavily contaminated, a single dilution may be sufficient.

Reference:

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater.* 21st ed. Washington, DC: American Public Health Association; 2005.