

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: E. Coli
Method: **IDEXX Colilert®-18 (MPN) (Aqueous)**

Per 40 CFR 136.3 Table 1 A footnote 17 and Table 1 H footnote 14, Colilert®-18 is recommended for E. Coli in marine water samples.

Equipment and Reagents:

Incubator capable of maintaining 35 ± 0.5°C	Quanti-Trays®: Specify type used. □ Quanti-Tray® □ Quanti-Tray®/2000	Sterile, non-buffered, oxidant-free dilution water
Quanti-Tray® Sealer	6-watt, 365-nm UV light	Anti-foam solution OR IDEXX Vessels with Anti-foam (optional)
Quanti-Tray® rubber insert	Sealer Check Dye	<i>E. coli</i> -strain (optional culture positive):
Comparator	Most Probable Number (MPN) Chart	<i>Pseudomonas aeruginosa</i> -strain (optional culture negative):
120 ml sterile vessels with Na ₂ S ₂ O ₃	Colilert®-18 reagent	<i>Klebsiella variicola</i> -strain (optional culture negative):

PLEASE COMPLETE CHECKLIST IN INDELIBLE INK

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

	GENERAL	LAB	SOP	EXPLANATION
1	Is the SOP reviewed at least every 2 years? What is the most recent review/revision date of the SOP? [15A NCAC 2H .0805 (a) (7)] Date:			Quality assurance, quality control, and Standard Operating Procedure documentation shall indicate the effective date of the document and be reviewed every two years and updated if changes in procedures are made. Verify proper method reference. During review notate deviations from the approved method and SOP.
2	Are all review/revision dates and procedural edits 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 using aseptic technique? [Colilert®-18, Procedural Notes]			Aseptic technique should always be followed when using Colilert-18.
5	Is residual chlorine neutralized at time of sample collection with sterile Na ₂ S ₂ O ₃ ? [40 CFR 136.3 Table II, Footnote 5]			
6	Are samples iced to above freezing but <10 °C 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.
7	Are samples checked for residual chlorine prior to analysis in the laboratory? [40 CFR 136.3 Table II] [15A NCAC 2H .0805 (a) (7) (M)]			Sample preservation shall be verified and documented. Use of TRC strips is allowed, See <i>Sample Collection, Preservation and Receipt Requirements Policy</i> .
8	What action is taken if chlorine is present? [15A NCAC 2H .0805 (a) (7) (M)] Answer:			If a laboratory receives a sample subject to G.S. 143-215.1 and 143-215.63 that does not meet sample collection, holding time, or preservation requirements, the laboratory shall document the incident, notify the sample collector or client, and secure another sample that meets the

				regulatory requirements, if possible. If another viable sample cannot be secured, the original sample may be analyzed but the results reported shall be qualified with the nature of the sample collection, holding time, or preservation infractions and the laboratory shall notify the State Laboratory of the infractions.
9	Are samples stored at <10 °C prior to analysis? [40 CFR 136.3 Table II]			
	PROCEDURE	L A B	S O P	EXPLANATION
10	Is Colilert-18 reagent stored at 2-25°C away from light? [IDEXX Colilert®-18, Storage]			Store at 2-25°C away from light.
11	Are samples brought to room temperature prior to analysis? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure]			Add contents of one pack to a 100-ml room temperature water sample in a sterile vessel.
12	If marine water is analyzed, are the samples diluted by at least tenfold prior to analysis? [IDEXX Colilert®-18, Procedural Notes]			Colilert-18 can be used for E. coli detection (but not coliforms) in marine water. Samples must be diluted at least tenfold. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
13	Is the content of one pack added to a 100-ml sample in a sterile vessel? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure]			Add contents of one pack to a 100-ml room temperature water sample in a sterile vessel.
14	Is the sample shaken until the contents are dissolved? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure]			Cap vessel and shake until dissolved.
15	If foaming occurs after the addition of Colilert-18 reagent, what action is taken? [IDEXX Colilert®-18, Procedural Notes] Answer:			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).
16	Is sterile, non-buffered, oxidant-free dilution water used to make dilutions when required? [IDEXX Colilert®-18, Procedural Notes]			Use only sterile, non-buffered, oxidant-free water for dilutions.
17	Are sealed sample trays placed into the incubator within 8 hours of collection? [40 CFR 136.3 Table II, Footnote 22]			Sample analysis should begin as soon as possible after receipt; sample incubation must be started no later than 8 hours from time of collection.
18	If a water bath is used, 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.
19	Are sealed trays incubated at 35°C ± 0.5°C? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure]			Place the sealed tray in a 35±0.5°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.
20	Are samples incubated for 18-22 hours? [IDEXX Colilert®-18, Result Interpretation]			Colilert-18 results are to be read after 18 hours of incubation. However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 22 hours total) to allow the color and/or fluorescence to intensify. Positives observed before 18 hours and negatives observed after 22 hours are also valid. In addition, laboratories may incubate samples for additional time (up to 22 hours total) for their convenience.
21	Is the date and time that 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.
22	Are incubator temperatures documented at the time samples are placed into and taken out of the incubator? [15A NCAC 2H .0805 (a) (7) (I)]			Each day samples are placed into or removed from an incubator, oven, water bath, refrigerator, or other temperature-controlled device, the temperature shall be checked, recorded, dated, and initialed.

23	Is the date and time that 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.
24	Are samples read using a 6-watt, 365-nm UV light held within 5 inches of the sample in a dark environment with the light facing away from your eyes? [IDEXX Colilert®-18, Result Interpretation]			Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
25	If the original sample has some background color, is the inoculated Colilert-18 sample compared to a control blank of the same water sample? [IDEXX Colilert®-18, Procedural Notes]			If a water sample has some background color, compare inoculated Colilert-18 sample to a control blank of the same water sample.
26	Are the number of positive wells counted and recorded for large and small wells on a laboratory benchsheet? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure] [15A NCAC 2H .0805 (a) (7) (F) (xviii)]			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. 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.
27	Is the MPN Table provided with the trays used to obtain a Most Probable Number? [IDEXX Colilert®-18, Quanti-Tray Enumeration Procedure]			Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.
28	Is the dilution factor documented when sample dilutions are made? [15A NCAC 2H .0805 (a) (7) (F) (xiii)]			All laboratories shall use printable laboratory benchsheets. Certified Data shall be traceable to the associated sample analyses and shall consist of: the dilution factor, where applicable.
29	Are results multiplied by an appropriate dilution factor when sample dilutions are made? [IDEXX Colilert®-18, Procedural Notes]			Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
	QUALITY ASSURANCE	L A B	S O P	EXPLANATION
30	Is each lot of Colilert®-18 checked with one E. Coli strain and one non-E. Coli strain? [Recommended in Colilert®-18, Quality Control Procedures]			1. One of the following quality control procedures is recommended for each lot of Colilert-18: A. IDEXX-QC Coliform and E. coli: Escherichia coli, Klebsiella variicola, and Pseudomonas aeruginosa. B. Quanti-Cult Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. C. Fill three sterile vessels with 100 mL of sterile non-buffered oxidant-free water and inoculate with a sterile loop of ATCC5 strains, Escherichia coli ATCC 25922/WDCM 00013 or ATCC 11775/WDCM 00090, Klebsiella variicola ATCC 31488/WDCM 00206 or Klebsiella aerogenes ATCC 13048/WDCM 00175, and Pseudomonas aeruginosa ATCC 10145/WDCM 00024 or ATCC 27853. 2. Follow the Quanti-Tray Enumeration Procedure above. 3. Results should match the Result Interpretation table above.
31	What corrective action is taken if the results do not match the Result Interpretation table? [15A NCAC 2H .0805 (as) (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.
32	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.
33	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.

34	<p>What corrective action is taken if the seal is not adequate? [NC WW/GW LCB Quanti-Tray® Sealer Check Policy]</p> <p>Answer:</p>		<p>If dye is observed outside the wells, either perform maintenance or use another sealer.</p>
35	<p>Is reagent water testing being performed every twelve months, if applicable? [NC WW/GW LCB Bacteriological Reagent Water Testing Policy]</p>		<p>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.</p> <p>Maximum Acceptable Limits are:</p> <p>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</p> <p>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.</p>
36	<p>Does the laboratory analyze duplicate samples at a rate of 5%? [15A NCAC 2H .0805 (a) (7) (C)]</p>		<p>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.</p>
37	<p>How are results of samples analyzed in duplicate reported?</p> <p>Answer:</p>		<p>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.</p> <p>This also applies if more than one sample is analyzed in a day.</p>
38	<p>What is the acceptance criterion for duplicates? [15A NCAC 2H .0805 (a) (7) (A)]</p> <p>Answer:</p>		<p>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.</p> <p>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.</p>
39	<p>What corrective action does the laboratory take if the duplicate sample results are outside of established control limits? [15A NCAC 2H .0805 (a) (7) (B)]</p> <p>Answer:</p>		<p>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.</p>
40	<p>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)]</p>		<p>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</p>

			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.
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Additional Comments:

Inspector: _____ Date: _____

Result Interpretation:

Appearance	Result
Less yellow than comparator when incubated at 35°C ± 0.5°C.	Negative for E.Coli
Yellow and fluorescence equal to or greater than the comparator when incubated at 35±0.5°C.	Positive for E. Coli