NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION

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

Parameter: **Enterococci**
Method: **IDEXX Enterolert® (Aqueous)**

EQUIPMENT:

	Sterile, transparent, non-fluorescent, 100-ml vessels		Incubator, 41± 0.5ºC
	Quanti-Trays®: Specify type used. ☐ Quanti-Tray® ☐ Quanti-Tray®/2000		6-watt, 365-nm, ultraviolet lamp
			Most Probable Number (MPN) chart
	Qunati-Tray® sealer		
	Quanti-Tray® rubber insert		

REAGENTS:

	Enterolert® Reagent		Sterile, non-buffered, oxidant-free water

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. 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 bottles? [SM 9060 A-2006 (1)] [Enterolert-2015]			**22nd Edition SM 9060 A-2006**: Collect samples for microbiological examination in clean, sterile, nonreactive borosilicate glass or plastic bottles or pre-sterilized plastic bags appropriate for microbiological use. Sterilized as directed in section 9030 B 19 and 9040 **Enterolert Method:** Aseptic technique should always be followed when using Enterolert.
5	Is residual chlorine neutralized at time of sample collection with sterile $Na_2S_2O_3$? [40 CFR 136.3 Table II] [SM 9060 A-2006 (2)]			For sampling chlorinated wastewater effluents add sufficient $Na_2S_2O_3$ to a clean sterile sample bottle to give a concentration of 100 mg/L in the sample. In a 120-ml bottle, 0.1 ml of a 3% solution of $Na_2S_2O_3$ will neutralize a sample containing 15 mg/L residual chlorine. Sterile vessels purchased from IDEXX provide enough sodium thiosulfate to neutralize up to 10 ppm of chlorine.
6	Are samples iced to above freezing but <10 º 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 upon receipt in the lab? [40 CFR 136.3 Table II]			Use of TRC strips is allowed, See June 20, 2007 preservation and hold time memo.
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 Certification that a non-compliant sample was received and analyzed. The sample must be qualified with the nature of the infraction on the DMR or client report.
9	Are samples stored at < 10 º C prior to analysis? [40 CFR 136.3 Table II]			
	PROCEDURE – Sample Preparation	**LAB**	**SOP**	**EXPLANATION**
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 must be started no later than 8 hours from time of collection.
11	Are contents of media pack aseptically added to 100 mL of water sample in a sterile container? [Enterolert-2015]			Aseptic technique should always be followed when using Enterolert.
12	If diluting, is only sterile, non-buffered, oxidant-free water used for dilutions (marine samples are diluted at least 10X)? [Enterolert-2015]			Use only sterile, non-buffered, oxidant-free water for dilutions.
13	Is the container capped and shaken until dissolved after adding media? [Enterolert-2015]			Cap vessel and shake until dissolved.
14	Is the Quanti-Tray® opened according to instructions while avoiding touching the inside of the foil or tray. [Enterolert-2015] [Quanti-Tray/2000-2013]			Use one hand to hold a Quanti-Tray® upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray® so that the Quanti-Tray® bends toward the palm. Open the Quanti-Tray® by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray.
15	Is sample poured into Quanti-Tray®, avoiding contact with the foil tab? [Enterolert-2015] [Quanti-Tray/2000-2013]			Pour the reagent/sample mixture directly into the Quanti-Tray®, avoiding contact with the foil tab.
16	Is foam allowed to settle before sealing? [Enterolert-2015] [Quanti-Tray/2000-2013]			Allow foam to settle.
17	Is the Quanti-Tray® sealed correctly by using an IDEXX Quanti-Tray® Sealer? [Enterolert-2015] [Quanti-Tray/2000-2013]			Place the sample-filled Quanti-Tray® onto the rubber tray carrier of the Quanti-Tray® Sealer with the well side (plastic) of the Quanti-Tray® facing down to fit into the carrier.
	PROCEDURE- Sample Analysis	**LAB**	**SOP**	**EXPLANATION**
18	Are samples incubated for 24-28 hours at 41 ± 0.5°C? [Enterolert-2015]			Place the sealed tray in a 41±0.5°C incubator for 24 hours. Enterolert results are definitive at 24-28 hours. In addition, positives for enterococci observed before 24 hours and negatives observed after 28 hours are also valid.
19	Is the time samples are place 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.
20	Is the incubator temperature documented? [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.
21	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.
22	Are wells that fluoresce blue when exposed to 6-watt, 365-nm UV lamp five inches away from the sample in a dark environment considered positive for Enterococci? [Enterolert-2015]			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.

23	Are results determined by counting and recording the number of positive wells and referring to the appropriate IDEXX MPN table? [Enterolert-2015] [Quanti-Tray/2000- 2013] [15A NCAC 2H .0805 (a) (7) (F) (xviii)]			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.
24	Are results multiplied by dilution factors when sample dilutions are made? [Enterolert-2015]			
	QUALITY CONTROL	**LAB**	**SOP**	**EXPLANATION**
25	Are media protected from light and stored according to manufacturer's recommendations (2-30°C)? [Enterolert-2015]			Shelf Life: Up to 12 months at 2–30°C
26	Is the Quanti-Tray sealer checked monthly? (NC WW/GW 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.
27	What corrective action is taken if the seal is not adequate? (NC WW/GW Policy) **ANSWER:**			If dye is observed outside the wells, either perform maintenance or use another sealer.
28	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.
29	Is the suggested water blank used for comparison when interpreting results? [Enterolert-2015]			This is a suggestion in the Enterolert method if needed to aid in interpreting results.
30	Is one of the method-**recommended** QC checks performed for each new lot of media? [Enterolert-2015]			One of the following quality control procedures (A or B) is **recommended** for each lot of Enterolert: A. IDEXX-QC Enterococci2: *Enterococcus faecalis*, *Escherichia coli*, and *Streptococcus bovis*. B. i. For each of the American Type Culture Collection (ATCC)3 bacterial strains (*Enterococcus faecium* ATCC 35667*, Serratia marcescens* ATCC 43862, and *Aerococcus veridans* ATCC 10400), streak the culture onto labeled TSA or Blood Agar plates and incubate at 35±2°C for 18–24 hours. ii. For each bacterial strain, touch a sterile 1 µL inoculating loop to a colony and use it to inoculate a labeled test tube containing 5 mL of sterile deionized water. Close cap and shake thoroughly.

				iii. For each bacterial strain, take a 1 µL loop from the test tube and use it to inoculate a labeled vessel containing 100 mL of sterile deionized water. These are your controls. Follow the Quanti-Tray enumeration procedure with the controls.
31	Which bacterial strains are tested? Do the results match the Result Interpretation table? [Enterolert-2015] **ANSWER:**			Option A from above: Enterococcus faecalis – Blue Fluorescence Escherichia coli – Lack of Fluorescence Streptococcus bovis – Lack of Fluorescence Option B from above: Enterococcus faecium – Blue Fluorescence Serratia Marcescens – Lack of Fluorescence Aerococcus viridans – Lack of Fluorescence
32	What corrective action does the laboratory take if the QC results are not as expected? [15A NCAC 2H .0805 (a) (7) (B)] **ANSWER:**			Any time quality control results indicate an analytical problem, the problem must be resolved and any samples involved must be rerun if the holding time has not expired.
33	Does the laboratory analyze duplicate samples at a rate of 5%? [15A NCAC 2H .0805 (a) (7) (C)]			Except where otherwise specified in an analytical method, laboratories shall analyze five percent of all samples in duplicate to document precision. Laboratories analyzing fewer than 20 samples per month shall analyze one duplicate during each month that samples are analyzed.
34	What is the acceptance criterion for duplicates? [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. 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.
35	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)] **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.
36	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.

Additional Comments:

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Inspector: _____Date:_____