Revision Date: February 9th, 2021.

*The SOP must be read completely and followed by laboratory staff doing analysis.*

*\*\*\*\*If you are using this as a template, add in your specific lab name AND date AND any steps that are different.\*\*\*\* This is only a resource, your procedure may be different than the one Medford is using.*

1. **Summary**
	1. Colilert\*-18 either simultaneously detects total coliforms and *E. coli*; or fecal coliforms in water. It is based on IDEXX’s proprietary Defined Substrate Technology\*. When total or fecal coliforms metabolize Colilert-18’s DST\* nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert-18’s DST\* nutrient-indicator, MUG, the sample also fluoresces under a 6 watt, 365nm UV light after incubation at 35 ± 0.5°C for 18 hours and up to 22 hours. Colilert-18 can simultaneously detect these bacteria at 1 cfu/100 mL even with as many as 2 million heterotrophic bacteria per 100 mL present.
	2. Colilert-18 is in Standard Methods for the Examination Water and Wastewater, On-line and in the 20th, 21st & 22nd edition AWWA, APHA, WEF, 9223B
2. **Scope and Application**
	1. The Colilert-18 method can be applied to fresh waters, drinking waters, ground waters, reuse waters, wastewaters and marine waters. It can be used as a Presence/Absence test or quantification with the Quanti-Tray® system.
	2. Since there can be wide range of coliform levels in surface waters and wastewaters, dilutions can be used with this method for detecting and enumerating the actual level.
3. **Interferences**
	1. Some samples containing humic material may have an innate color and a control blank of the same water sample may be required for comparison to the inoculated sample or a dilution may be made.
	2. Heterotrophic bacteria greater than 2,000,000/100mL could yield a positive coliform reaction for coliforms.
4. **Equipment and Supplies**
	1. Sterile vessels, glass or plastic (free from fluorescence) with or without sodium thiosulfate. Any water containing an oxidizing agent such as chlorine must be neutralized with sodium thiosulfate.
	2. Vessels containing sodium thiosulfate must neutralize up to 15 mg/L of chlorine for wastewater effluent samples.
	3. Vessels must be at least 120 mL or of larger capacity to hold 100 mL sample to allow for proper mixing of sample.
	4. 51 well Quanti-Tray or Quanti-Tray/2000
	5. Quanti-Tray Sealer
	6. Incubator maintained at 35 ± 0.5°C.
	7. 6-Watt 365nm UV light.
5. **Reagents and Standards**
	1. Sterile, non-buffered, oxidant-free water for dilutions.
	2. 51 Well Quanti-Tray or Quanti-Tray 2000 Comparator.
	3. Antifoam reagent (optional).
	4. Sterile vessels or sterile vessels containing sodium thiosulfate to neutralize up to 15mg/L chlorine.
	5. Store Colilert-18 at 2-25°C away from light. The expiration date is indicated on the package (15 months from the date of manufacture).
6. **Sample Collection, Preservation, and Storage**
	1. Sampling procedures as described in detail in the Standard Methods for the Examination of Water and Wastewater.
	2. Storage Temperature and Handling Conditions: Ice or refrigerate bacteriological samples at a temperature less than 10°C (2-10°C) during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Ensure that sample vessels are not totally immersed in water during transit. Do not allow samples to freeze. If frozen, sample cannot be thawed, and a new sample is required.
	3. For wastewater for compliance, do not exceed 8 hours from time of collection to incubation. Collect samples in designated clean plastic bottles.
7. **Quality Control**
	1. Check the temperature of the incubator at least twice per day (when in use) separated by at least 4 hours to insure it is within the stated limits. Record the date, temperature, time of reading and initial.
	2. Check thermometers at least annually against NIST certified thermometer.
	3. Quality control should be conducted on each lot of Colilert-18 initially and furthermore a 90 day recheck. The following quality control procedure is recommended (but not required) for each lot of Colilert-18 when used for total coliform and *E. coli* testing: IDEXX-QC Coliform and *E. coli*: Consists of 3 each of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.
	4. See the package insert for instructions.
	5. Follow the Quanti-Tray Enumeration Procedure below
	6. Alternatively, certificates of analysis, MSDS and other resources can be gathered specific to the LOT# at www.idexx.com/en/water/
8. **Quanti-Tray Sealer Procedure**
	1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.
	2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm.
	3. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray.
	4. Pour the reagent/sample mixture directly into the Quanti-Tray, avoiding contact with the foil tab. Allow foam to settle (or use IDEXX Anti-Foam solution)
	5. 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
	6. Seal according to the Quanti-Tray Sealer instructions.
	7. Incubate according to reagent instructions.
9. **Sample Analysis Procedure**
	1. Carefully separate one blister pack from the strip taking care not to accidentally open the adjacent pack.
	2. Ensure the powder is in the bottom of the blister pack.
	3. Hold the blister pack face down (paper side up) at the top and towards the bottom and snap back at the score line forming a “V”, with the opening facing into the open vessel.
	4. Allow the powder to fall into the water sample contained in the sterile, nonfluorescent vessel.
	5. Aseptically cap and seal the vessel.
	6. Mix well until dissolved.
	7. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
	8. Place the sealed tray in a 35±0.5°C (or 44.5±0.2°C for fecal coliforms) incubator (prewarming to 35°C is not required).
	9. Incubate for 18 and up to 22 hours at 35 ± 0.5°C.
	10. For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.
	11. Read results according to the Result Interpretation table below.
10. Result Interpretation

|  |  |
| --- | --- |
| **Appearance** | **Result** |
| Less yellow than the comparator | Negative for total coliforms and *E. coli* |
| Yellow equal to or greater than the comparator | Positive for total coliforms |
| Yellow and fluorescence equal to or greater than the comparator | Positive for *E. coli* |

* 1. If yellow is observed, check vessel for fluorescence by placing a 6-watt, 365nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If the fluorescence is equal to or greater than the comparator, the presence of *E. coli* is confirmed.
	2. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.
1. Waste Management
	1. Medford WWTP laboratory shall do it’s best to minimize pollution of the environment and manage its hazardous wastes in a safe and environmentally sound matter.
	2. Consider environmental impact when purchasing materials, handling chemicals and disposing of wastes.
2. Safety Plan

Handling microbial hazards requires proper protection equipment and measures.

* 1. PPE:

Safety Glasses

Nitrile or Latex examination gloves

Hood Vent (if needed while making bleach solution or disinfecting contaminated and negative samples.)

* 1. Procedure:

 Lab staff should disinfect set-up area prior to and after handling samples. Using a 1:10 dilution of bleach and water, spray and wipe down area. Carefully handle samples to minimize contamination or spills. When set-up is complete, spray the area and wipe up using a clean paper towel. It is recommended that staff wash their hands using an antibacterial soap even after using gloves. Staff shall also follow this procedure while disinfecting and disposing samples.

**References**

* 1. IDEXX Colilert-18 Test Kit Insert
	2. Quanti-Tray Insert and Most Probable Number (MPN) Table
	3. Chapter V Critical Elements for Microbiology
	4. Standard Methods for the Examination Water and Wastewater 22nd Edition

SOP Prepared by:

Brooke Klingbeil

Laboratory Director

Medford Wastewater Treatment Plant

Phone: (715)748-4122

E-mail: bklingbeil@medfordwi.us

Fax: (715)748-0822

I have read this SOP and will perform lab work as specified within this procedure. (sign and date)

