STANDARD OPERATING PROCEDURE FOR *ESCHERICHIA COLI (E. COLI)* AND TOTAL COLIFORM QUANTIFICATION USING THE IDEXX QUANTI-TRAY/2000 SYSTEM

State of Utah
Department of Environmental Quality
Division of Water Quality

Revision 1.2
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Utah Division of Water Quality (DWQ) Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical experts. This document is intended primarily for internal DWQ use. This SOP should not replace any official published methods.

Any reference within this document to specific equipment, manufacturers, or supplies is only for descriptive purposes and does not constitute an endorsement of a particular product or service by the author or by DWQ. Additionally, any distribution of this SOP does not constitute an endorsement of a particular procedure or method.

Although DWQ will follow this SOP in most instances, there may be instances in which DWQ will use an alternative methodology, procedure, or process.[1]

[1] *Disclaimer language above adapted from Washington State Department of Ecology SOPs.*

REVISION PAGE

Date	Revision #	Summary of Changes	Sections	Other Comments
6/15/12	1	Not applicable	Not applicable	Previous version called Version 4, dated 3/28/11 was put into the new format. Added 18-hr Colilert test option. Made minor general edits. Began document control/revision tracking.
6/27/13	1.1	1) Added the option of watching the training video 2) Suggest internal thermometer for thermototes 3) Wash hands before sample processing. 4) Removed limit for stacking trays. 5) Analyze a dilution blank. 6) Only trays are considered infectious. 7) DOC form and Bench Sheet were updated and simplified.	1) 7.0 2) 9.1 3) 9.2 4) 9.4 5) 9.6 6) 9.8 7) Appendices	1) For experienced samplers only
5/1/14	1.2	Minor formatting	various	

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1.0 SCOPE AND APPLICABILITY

This document presents the Utah Division of Water Quality's (DWQ) Standard Operating Procedure (SOP) for enumeration of *Escherichia coli (E. coli)* and total coliforms in water samples using the IDEXX Quanti-Tray/2000 System. This SOP covers sample processing and analysis only; it does not cover water sample collection. Collection of water samples for *E. coli* analysis is discussed in DWQ's SOP for Collection and Handling of *Escherichia coli (E. coli)* Samples. This SOP applies to all personnel processing/analyzing *E. coli* samples including DWQ monitors, non-DWQ cooperators, and volunteer monitors.

The IDEXX Colilert® Quanti-Tray/2000 method is an enzyme-substrate, MPN-based, EPA-approved method for quantifying total coliform and *E. coli* bacteria in water samples. The detection limit for this test ranges from 1 Most Probable Number (MPN) per 100 ml of sample to >2419.6 MPN per 100 ml of sample. Although this method is suitable for use with both surface water and drinking water samples, this SOP focuses on analysis of surface water samples collected from Utah's lakes, reservoirs, rivers, and streams.

E. coli concentrations are used in water quality assessments and health advisories of Utah's recreational waters. Refer to Utah's Integrated Report for further explanation of Utah's *E. coli* water quality standards for recreational waters, tiered rotating basin schedule, sampling site selection, and assessment methodology. For more information regarding Utah DWQ's *E. coli* Program, contact the Bacteriological Program Coordinator:

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2.0 SUMMARY OF METHOD

Surface water samples are collected in sterile 120 ml polypropylene bottles containing sodium thiosulfate and stored on wet ice or ice packs until processing. Samples must be processed and in the incubator within 8 hours of collection. One packet of Colilert reagent (original 24-hour formula) is poured into each sample bottle and shaken gently to dissolve. Samples are transferred to 97-well Quanti-Trays/2000 and sealed using the Quanti-Tray sealer. The samples are incubated at 35 ± 0.5°C for 24 -28 hours. Alternatively, the Colilert-18 reagent may be used to achieve results in 18-22 hours. A color change in tray wells from clear to yellow observed under ambient lighting indicates the presence of total coliform bacteria. Fluorescence under UV lighting of the same

yellow wells indicates presence of *E. coli*. Counts of small and large yellow and fluorescence wells are used in conjunction with the IDEXX MPN table to determine number of each type of bacteria. Alternatively, the "MPN Generator" software provided by IDEXX can be used to calculate MPN values. Results are reported as MPN/100 ml.

3.0 DEFINITIONS

aseptic technique: performed under sterile conditions

E. coli: *Escherichia coli.* A type of bacteria belonging to the fecal coliform group of bacteria found naturally in the gut and feces of warm blooded animals. Most *E. coli* strains are harmless, but a few can cause disease in humans. *E. coli* is a truer indicator of fecal contamination than total coliforms or fecal coliforms. *E. coli* is easier and less expensive to detect than actual pathogens and is used as an indicator for the potential presence of pathogens associated with fecal contamination.

ml: milliliters

MPN: most probable number

QA/QC: quality assurance and quality control

SAP: sampling and analysis plan

Total coliform: Rod-shaped gram-negative bacteria which ferment lactose and contain the enzyme β-D-galactosidase. They are abundant in the feces of warm-blooded animals but also include bacteria that are naturally present in the soil and water environment. They are not the cause of sickness, but their presence is used to indicate potential for contamination of drinking water.

UV: ultraviolet

4.0 HEALTH AND SAFETY WARNINGS

Samples could contain pathogenic microorganisms. Personnel who collect and/or process the samples should protect themselves from waterborne illnesses by wearing clean disposable gloves and/or washing their hands frequently.

Use caution when using the Quanti-Tray Sealer as it might be hot.

When opening the Colilert reagent snap pack, open the pack so that the pack is facing away from you. Note: The Colilert reagent is not hazardous according to the manufacturer's MSDS but inhaled powder may cause lung irritation.

Wear safety glasses and do not look directly into the UV light.

5.0 CAUTIONS

Avoid sample contamination. Do not touch the inside, lip, or cap of the sampling container or Quanti-Tray.

Sample bottles must contain at least 100 ml of sample or the Quanti-Tray may not fill completely, resulting in invalid results.

6.0 INTERFERENCES

Samples may contain material that affects the color of the sample. If this situation does arise, compare inoculated trays to a control tray containing only water.

Test sensitivity maybe affected by taking the samples out of the incubator too soon, resulting in false negatives. Samples must be incubated for the full term.

Autofluorescent plasticware or glassware may produce false positives. Check sample containers prior to sampling and processing.

Samples shaken too vigorously may foam and result in trays that are difficult to read/interpret; avoid excessive foaming of the sample before pouring it into the tray.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

Samplers/processors are required to read this SOP annually and acknowledge they have done so via a signature page (see **Appendix 1**) that will be kept on-file at DWQ along with the official hard copy of this SOP. New sample processors must also complete an initial Demonstration of Capability (DOC) for the Colilert method (see **Appendix 2**).

Personnel performing water sampling must be familiar with sampling techniques, safety procedures, proper handling, and record keeping. Samplers are responsible for attending refresher meetings and/or video trainings held each spring/summer to review procedures and techniques. New staff must be trained initially by DWQ unless otherwise approved by the Bacteriological Program Coordinator.

8.0 EQUIPMENT AND SUPPLIES

Note: All Colilert supplies are purchased through IDEXX. Be sure to check expiration dates before using.

_____Copy of this SOP

_____Site portfolio or copy of project-specific SAP

_____Incubation data sheets (bench sheets), see **Appendix 3**

_____Quanti-Trays/2000 [IDEXX cat# WQT-2K (100 trays)]

_____Colilert Comparator (IDEXX cat# WQT2KC)

_____Colilert Reagent [IDEXX cat# WP020I (20-pack), #WP100I (100-pack), or #WP200I (200-pack)]

OR,

_____Colilert-18 Reagent [IDEXX cat# WP020I-18 (20-pack), #WP100I-18 (100-pack), or #WP200I-18 (200-pack)]

_____Sealer (IDEXX cat# WQTS2X-115) and Rubber insert for sealer (IDEXX cat# WQTSRBR-2K)

_____Incubator and power supplies (Note: Programmable temperature is strongly recommended)

_____Incubator thermometer

_____Handheld 6-watt long wave UV lamp: 115 volts (Spectronics Corporation Cat # 1608994)

_____Sterile, disposable pipets (1 mL to 25 mL) for performing dilutions

_____Pipettor for performing dilutions

_____Sterile 120-ml polystyrene bottles containing sodium thiosulfate [e.g. IDEXX cat# WV120SBST-20 (20 pack) or WV120SBST-200 (200 pack)] for making up diluted samples

_____Autoclavable Biohazard Bags (VWR Cat #14220-086)

_____Elastic closures for biohazard bags (Fisher Scientific #D18158)

_____Sharpie and Pen/Pencil

_____Deionized/distilled water (preferably sterile)

_____MPN Generator software or MPN Table

If collecting samples, the following supplies are also needed:

_____Copy of DWQ's SOP for Collection and Handling of *E. coli* Samples

_____Site portfolio or copy of project-specific SAP

_____Field data sheets or project-specific field sheets

_____Water-proof pens/markers

_____Sterile 120-ml polystyrene bottles containing sodium thiosulfate [e.g. IDEXX cat# WV120SBST-20 (20 pack) or WV120SBST-200 (200 pack)]

_____Deionized/distilled water (preferably sterile)

_____Maps

_____GPS unit

_____Camera

_____Cooler

_____Wet ice or ice packs

_____Safety gear (especially gloves and/or hand sanitizer)

_____Chest waders with belt or hip boots

9.0 PROCEDURE

For sample collection, see DWQ's SOP for the Collection and Handling of E. coli Samples. Note: 100 ml of surface water must be collected. Do not over or under fill bottles. The amount of powder in each reagent snap pack is meant for a 100 ml sample +/- 2.5% vessel volume[2]. Samples should be stored in a cooler on ice or in a refrigerator at 4°C until processing. Samples must be processed and placed into the incubator within 8 hours of collection.

Figure 1 provides an overview of the Colilert method for enumeration *E. coli* in water samples.

9.1 Preliminary Procedures

1) Make sure the incubator is turned on.

2) Immediately prior to processing the samples, verify that the temperature of the incubator is 35 ± 0.5°C preferably using the internal thermometer. Use external display if no internal thermometer is available.

3) Turn on the Quanti-Tray Sealer and allow it to warm up. An orange light indicates the power is on; a green light indicates the sealer is ready to use.

4) If using a portable incubator, warm samples in a 35°C water-bath for 30 minutes before proceeding. Check the temperature on the incubator. The temperature must be set for 35 ± 0.5°C. Check frequently to ensure constant temperature. To ensure proper temperature during incubation period, store incubator in a location not likely to experience fluctuations in ambient temperature. An internal thermometer is also recommended when using a portable incubator.

[2] Reference - Personal communication with IDEXX employee Krista Doucette

9.2 Sample Preparation

1) Wash your hands.

2) Label the trays with the sample location, incubation date and start time, initials of person processing, and replicate number (if applicable). Make sure the information on each sample bottle correctly matches the labeled tray. Be sure to include trays for QC samples.

3) One Colilert Reagent snap packet is required per 100 ml water sample. Separate one snap pack from the Colilert Reagent strip. Tap to ensure the medium is in the bottom of the pack.

4) Aseptically snap open the pack and transfer it to one 120 ml sample bottle containing sample. Make sure to face the snap pack away from you before opening. Shake or swirl the sample bottle gently to dissolve the powder, trying not to cause excessive foaming. Make sure any foam settles before continuing.

9.3 Colilert Quanti-Tray and Sealer

1) Use one hand to hold a tray upright and squeeze the upper part so that it opens and pull the foil gently away from the tray using the tab. Pour the sample/reagent solution directly into the tray using aseptic techniques. Tap the tray gently to dislodge any air bubbles inside the wells.

2) Place the tray into the rubber insert with the well side facing down.

3) Feed the rubber insert into the sealer with the open end of the tray facing away from the sealer.

4) Remove the sealed tray from the back of the sealer.

5) Repeat for all samples including QC samples.

9.4 Incubation

1) Fill out the bench sheet (**Appendix 3)** including the minimum information: the sample location, start time and date of incubation, start and end temperature of incubation, and initials of person processing.

2) Invert the sealed trays and incubate at 35 ± 0.5°C for 24 hours. If using Colilert-18, incubate trays for 18 hours. Use caution when stacking trays so as to not to block the airflow in the incubator.

3) Repeat this process for all samples and QC samples.

4) Record the temperature of the incubator and the time on the bench sheets at the start and end of incubation.

5) Retain bench sheets for 3 years. They do not need to be sent to DWQ unless requested by DWQ.

9.5 Interpretation

1) When reading the trays, contrast the wells with the Colilert Comparator which shows the lowest level of yellow and fluorescence that is considered positive for total coliform and *E. coli* counts.

2) Count the number of large and small wells that are yellow under normal lighting. Record these counts on the back of the tray. The yellow color is indicative of the presence of total coliforms.

3) If a well is yellow but lighter than the Comparator or if you are uncertain if the color is yellow, then incubate the tray for up to 4 more hours (a total of 28 hours). Read the tray again. If the same color intensifies, it is considered positive for total coliforms. If the color does not intensify, then it is negative.

4) If yellow wells are present, check those same wells for fluorescence by using the UV light. Hold the UV light 5 inches from the tray (turn out the ambient lights for best results). Do not look directly at the light or wear safety glasses to protect your eyes from the UV light. If the fluorescence is equal to or greater than that of the Comparator, count and record the number of blue fluorescent wells, both large and small. Blue fluorescent wells (that were also yellow) are considered positive for *E. coli*.

5) The field blank should remain colorless and negative for fluorescence throughout the 24 hours.

6) *Interpreting empty wells:* When a tray has exactly 100 ml of sample and all 48 wells are full there should be a little less than 2 ml in the top overflow well.

   a) Any well that is partially full is interpreted as seen and an empty well is considered negative[3]. Having up to 2 empty wells will not result in a statistically significant difference in measured total coliform/*E. coli* concentrations.

   b) If more than 2 wells are empty, inadequate sample volume was collected and the sample should be considered invalid.

   c) If all wells are positive except for one empty well, then the empty well should be considered positive, as a conservative measure.

[3] Reference - Personal communication with IDEXX employee Krista Doucette

9.6 Dilutions

1) There are situations when the numerical value from the bacteriological analysis needs to be an actual number (e.g. for TMDL investigations). Thus samples that exceed the threshold, those with concentrations > 2419.6 MPN/100 ml, should be diluted before analysis takes place when possible.

2) A knowledge of the source water to be sampled, seasonal variability, storm events, or known influences can be helpful for understanding when dilutions need to be made.

3) If it is unknown if or how much a sample needs to be diluted, take two samples at the sampling site. Analyze one sample undiluted and analyze the second sample at a 1:10 dilution.

4) Prepare a 1:10 dilution by thoroughly mixing the sample and then pipetting 10 ml of sample into a new unused sample bottle using a 10 ml sterile disposable pipette. Next, use distilled/deionized water to bring the volume up to the 100 ml line. Mix gently to homogenize. Process the sample as you would a regular sample. Be sure to keep track of the diluted sample by labeling the tray and filling out the bench sheet accordingly.

5) Prepare a dilution blank by analyzing 100 ml of the distilled/deionized water used for making dilutions as a regular sample.

6) When reporting the results from diluted samples remember to multiply the results by the appropriate dilution factor (i.e. multiply the results from a 1:10 diluted sample by 10 to get the final result in MPN/100 ml).

9.7 Data Analysis and Calculations

The determination of MPN/100 ml for both total coliforms and *E. coli* can be done in one of two ways:

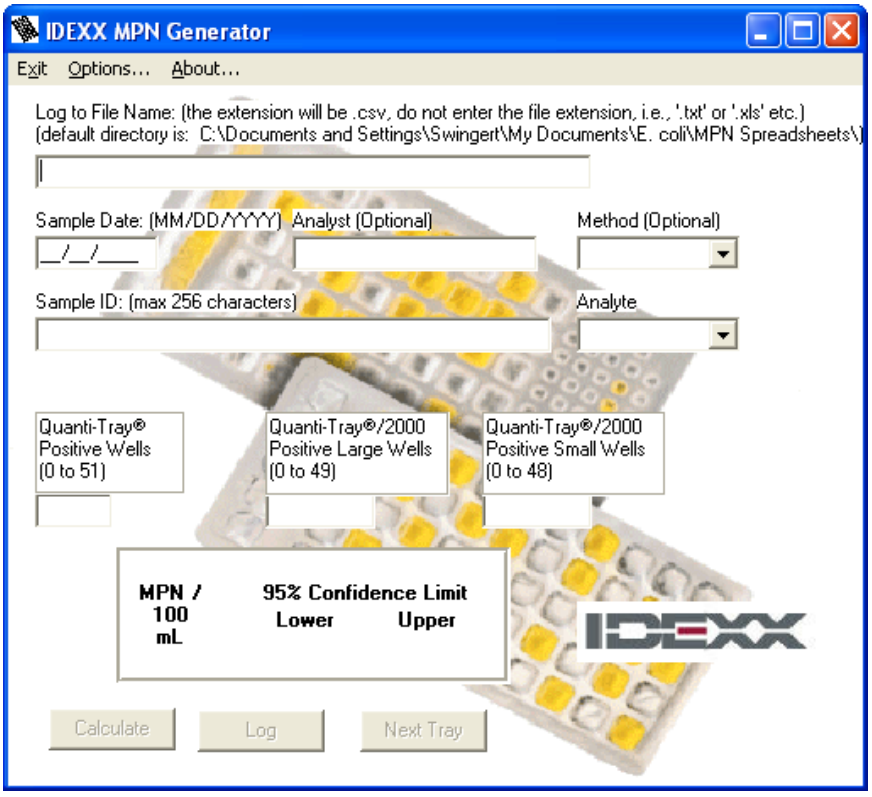
1) Use the MPN tables.

2) Use the IDEXX MPN Generator. This is the preferred method.

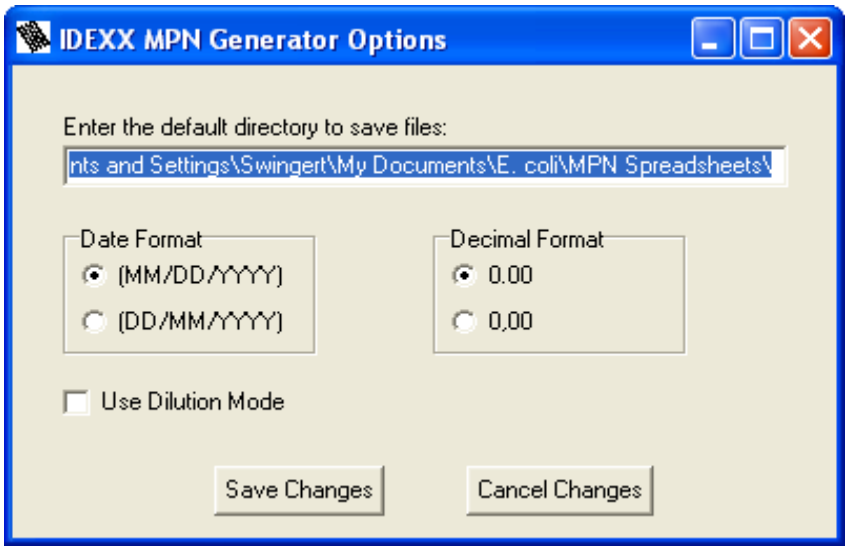
   a) Download the Generator from IDEXX's website.
   http://www.idexx.com/view/xhtml/en_us/water/mpn-generator.jsf

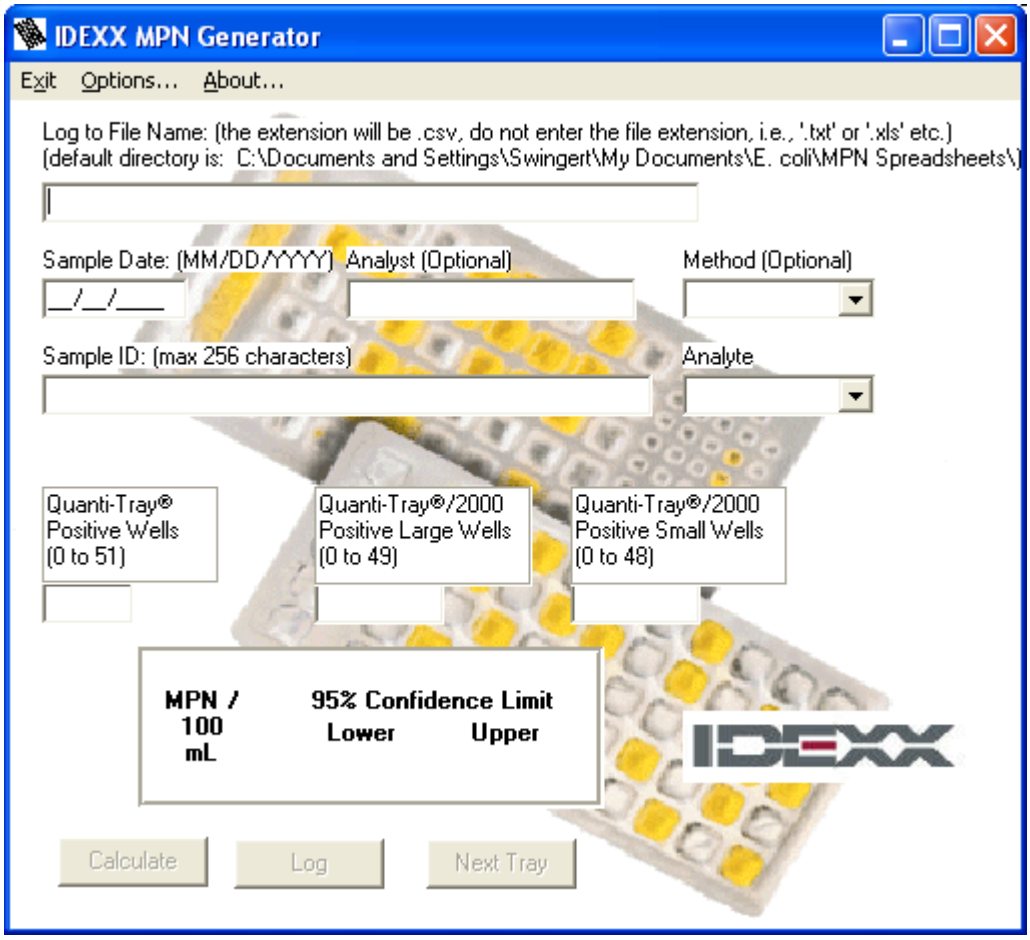
   b) Open the Generator and follow the directions below.

      i. Click "Options"



ii. Enter the file directory of where you want your MPN spreadsheet to be stored. Then click "Save Changes". This is also where you can choose to run the Generator in "Dilution Mode" if a diluted sample was analyzed.





iii. Enter your sample date, analyst, and method (Colilert or Colilert-18). Type in the name of your Sample ID (Ex. 4992510 *E. coli*). Note: You might want to label which sample is for your *E. coli* and total coliforms here. Choose which analyte (either total coliforms or *E. coli*) you are calculating. Skip the box for "Quanti-Tray Positive Wells (0 to 51)". This is for a different method.

iv. For Total Coliforms, choose total coliforms for your analyte. Enter the number of large yellow (positive) wells in the "Quanti-Tray/2000 Positive Large Wells (0-49)" space and the number of small yellow wells in the "Quanti-Tray/2000 Positive Small Wells (0-48)" space. Click on the "Calculate" button. The results are recorded in MPN/100 ml. Record these results on the bench sheet. The MPN Generator also gives you the 95% confidence limits.

v. Click on the “Log” button to store these results in the automatically created spreadsheet.

vi. For *E. coli*, choose *E. coli* for your analyte. Enter the number of large wells that are both yellow and fluorescent in the “Quanti-Tray/2000 Positive Large Wells (0-49)” space and the number of small wells are both yellow and fluorescent in the “Quanti-Tray/2000 Positive Small Wells (0-48)” space. Click on the “Calculate” button. The results are recorded in MPN/100 ml. Record these results on the bench sheet. The MPN Generator also gives you the 95% confidence limits.

vii. Click on the “Log” button to store these results in the automatically created spreadsheet.

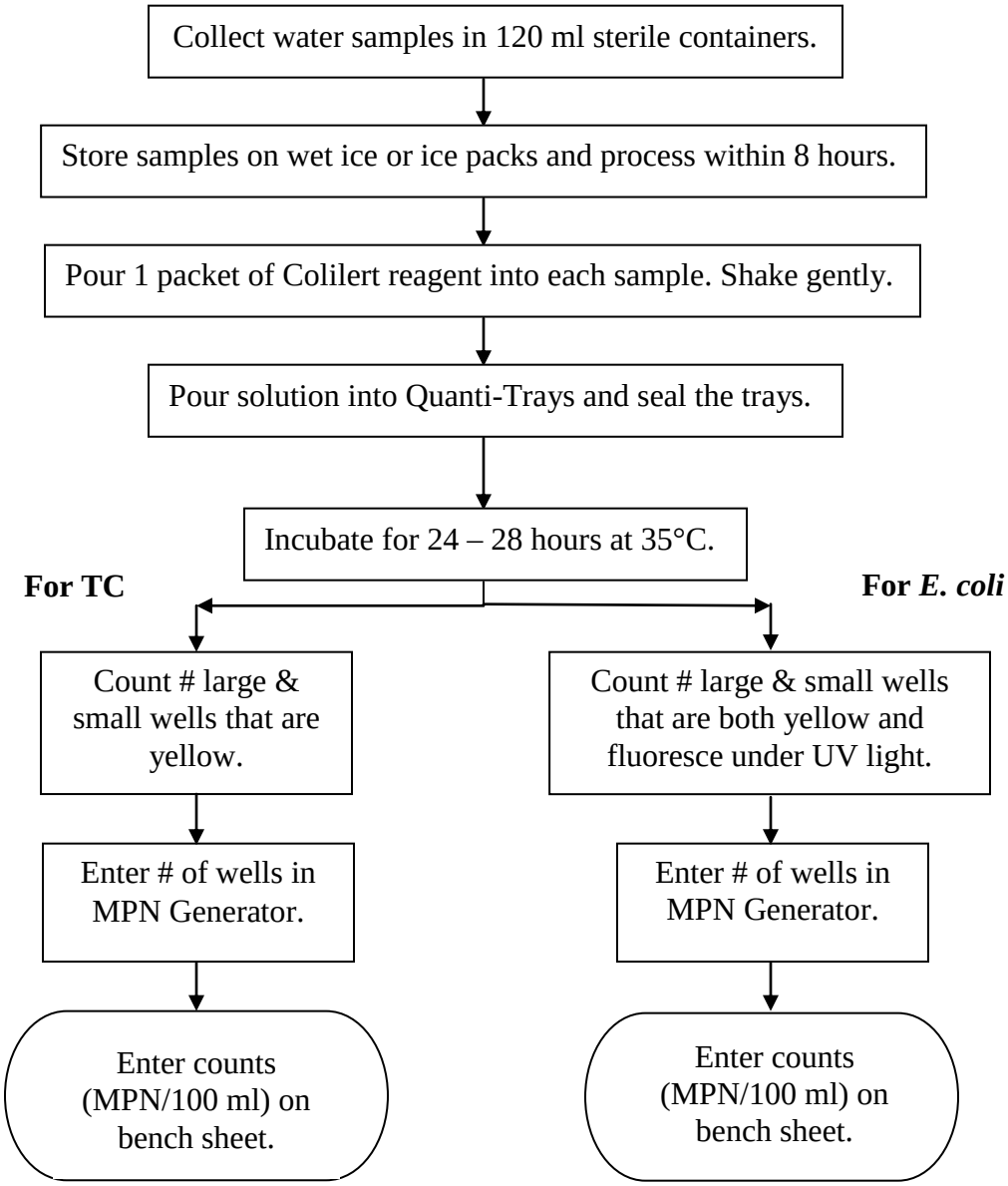
viii. Note on QC Samples: Field blanks should be labeled “Blank” for the sample location. Duplicate samples should be labeled “DUP” on the sample location.

The blank samples should have no detectable *E. coli* (<1 MPN/100 ml). If the blank is positive for *E. coli*, make an attempt to determine the source of contamination (e.g., Go back and retest deionized/distilled water, call DWQ to discuss, etc.).

9.8 Disposal

Sample bottles, empty snap packs, and pipettes are not considered infectious and may be thrown in the trash. Place all used trays in an autoclavable biohazard bag and autoclave for 30 minutes before disposal into municipal trash. Bags may be brought to DWQ for disposal but must be transported in a leak-proof container, preferably a plastic bin that can be bleached after use.

Figure 1. Overview of *E. coli* and total coliform quantification.



10.0 DATA AND RECORDS MANAGEMENT

Data for regular samples, field blanks, and duplicates should be submitted monthly to the Bacteriological Program Coordinator via an emailed excel file containing at a minimum the Monitoring Location ID (MLID, formerly STORET) or GPS coordinates and site name, date, collector's initials, MPN for total coliforms, MPN for *E. coli*, replicate #, and dilution factor (if applicable).

If using the MPN Generator, the automatically-generated spreadsheet may be sent directly to the Bacteriological Program Coordinator if it contains all of the sample metadata listed above.

Be sure to record results on bench sheets. Before sending data to DWQ, cross-check bench sheets and excel files. Retain bench sheets for 3 years but do not send to DWQ unless requested.

As instructed in DWQ's SOP for Collection and Handling of *E. coli* Samples, scan-to-PDF, fax, or mail photocopies of the field sheets to DWQ at the end of each recreation season. Retain the original field sheets in your records. Be sure that sample ID's, dates, and times between field sheets, data files, and bench sheets are consistent.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

1) Each analyst must read this SOP annually and date and sign the form kept at DWQ with the applicable SOP revision hardcopy.

2) Each new analyst should complete an initial Demonstration of Capability (DOC) to detect and enumerate *E. coli* by the approved Colilert method and should watch the training video every year thereafter.

3) Record the temperature of the incubator using the internal thermometer (or external digital display if no internal thermometer is available) when the samples are initially placed inside the incubator and when the samples are removed.

4) Maintain the incubator temperature at 35 ± 0.5°C for the duration of incubation.

5) Verify proper sample collection, handling, and analysis by testing field blanks and field duplicates. Prepare field blanks with distilled/deionized water at the beginning of each sampling day. Test for duplicates at a frequency or 10%. See further details in DWQ's SOP for Collection and Handling of *E. coli* Samples.

6) Preferably use the same deionized/distilled water source for field blanks and dilutions (to capture potential for false positives during dilution preparation) or perform dilution blanks unless processing samples at a laboratory where deionized/distilled water is analyzed routinely for contamination.

7) Use media within the expiration date. Store media in a cool, dark place.

8) Store Comparator in a dark place and replace if expired.

9) Check thermometers annually against NIST-certified thermometer and replace if the difference is greater than 1°C. DWQ has an NIST-certified thermometer.

10) It is recommended that lot number of media, vessels, and trays be recorded in a logbook so that they can be traced back to a batch of tested samples.

12.0 REFERENCES

IDEXX Product Inserts

DWQ's Integrated Report
(http://www.waterquality.utah.gov/WQAssess/documents/IR2010/Part1/2010_Part-1-IR-Final_10Nov2010.pdf)

IDEXX MPN Table or MPN Generator
(http://www.idexx.com/view/xhtml/en_us/water/mpn-generator.jsf)

User Manual, Quanti-Tray Sealer. IDEXX Laboratories, Inc., Westbrook, Maine.

User Manual, Quanti-Tray/2000. IDEXX Laboratories, Inc., Westbrook, Maine.

Standard Methods for the Examination of Water and Wastewater. 20th edition. Edited by Clesceri *et a*l.

Related DWQ SOPs:

Standard Operating Procedure for Collection and Handling of *Escherichia coli* (*E. coli*) Samples

13.0 APPENDICES

Appendix 1 – SOP Acknowledgment Form

This SOP must be read and this form signed annually. This form must be kept with the current version of the SOP.

Document Title:	
Document Revision Number:	
Document Revision Date:	

Please sign below in accordance with the following statement: "I have read and understood the above referenced document. I agree to perform the procedures described in this SOP in accordance with the document until such time that it is superseded by a more recent approved revision."

Printed Name	Signature	Date

Appendix 2 – DOC Form

Initial Demonstration of Capability (DOC) Form for Bacteriology Testing

Analyst Name (print):	
Analyst Signature:	
Date of DOC:	
Analytical Method(s):	IDEXX Colilert with Quant-Tray/2000

Sample ID	**Number of Large Yellow Wells**	**Number of Small Yellow Wells**	**Number of Large Fluorescing Wells**	**Number of Small Fluorescing Wells**	***E. coli* Concentration MPN/100 ml**	*LEAVE BLANK; THIS COLUMN FILLED OUT BY TRAINER* **True Result MPN/100 ml**

☐ **PASS** ☐ **FAIL**

Trainer Approval (print):	
Trainer Signature:	
Approval Date:	
Mgt Approval (print):	
Mgt Signature:	
Approval Date:	

Appendix 3 – Bench Sheet

Colilert Quanti-Tray/2000 Method Bench Sheet

Project/Run:	
Analyst:	
Date and Start Time:	
Date and End Time:	
Start Temp:	
End Temp:	
Colilert 24-hr or 18-hr?	

Sample ID	# Lg Yellow Wells	# Sm Yellow Wells	# Lg Fluor. Wells	# Sm Fluor. Wells	TC (MPN/100ml)	*E. coli* (MPN/100ml)	Dilution Factor	*E. coli* corrected for dilution (MPN/100ml)