STANDARD OPERATING PROCEDURE FOR COLLECTION, HANDLING AND QUANTIFICATION OF TOTAL COLIFORM AND *ESCHERICHIA COLI (E. COLI)* SAMPLES

WATER QUALITY

State of Utah
Department of Environmental Quality
Division of Water Quality

Revision 2.2
Effective May 2020

Foreword

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 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.

The methodology detailed below is the protocol followed by DWQ's monitoring staff and verified by DWQ's Quality Assurance officer.

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06/02/2020

Date

Toby Hooker
Toby Hooker (Jun 2, 2020 16:45 MDT)

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06/02/2020

Date

Revision Page

Date	Revision	Summary of Changes	Sections	Other Comments
1/30/2020	2.0	Created Combined SOP	ALL	Combined collection and analysis SOP's into one.
2/4/2020	2.1	Updated QA/QC measures	2, 9, 11	Standardized equipment blank usage. Updated language concerning duplicate, replicate, and triplicate.
2/10/2020	2.2	Updated language, grammar, and structure	ALL	Clarified and revised sentence structure and grammar throughout the entire document. Added Quick Sampling Guide. Updated field data sheet. Reformatted signature pages.

Table of Contents

1.0 SCOPE AND APPLICABILITY

This document presents the Utah Division of Water Quality's (DWQ) Standard Operating Procedure (SOP) for water sample collection from Utah's lakes and rivers/streams for *Escherichia coli* (*E. coli)* analysis. This SOP applies to all personnel collecting *E. coli* samples including DWQ monitors, DWQ cooperators, and volunteer monitors.

The IDEXX Colilert® Quanti-Tray®/2000 method is an enzyme-substrate, Most Probable Number (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 (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 of Utah's recreational waters in addition to informing recreational health advisories. For further explanation of Utah's *E. coli* water quality standards for recreational waters and assessment methodology refer to Utah Administrative Code R317-2 and project specific Sampling and Analysis Plans (SAP) or other planning documents. For more information regarding Utah DWQ's *E. coli* Program, contact the *E. coli* Monitoring Program Coordinator:

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2.0 SUMMARY OF METHOD

Water samples (minimum 100-mL volume) are collected in sterile 120 mL polypropylene bottles using grab sampling techniques. Sampling bottles contain sodium thiosulfate to neutralize chlorine in wastewater outfalls or streams for which baseflow is largely provided by wastewater effluent. Lake/reservoir samples are collected using a transect sampling procedure and river/stream samples are collected in one bottle unless multiple samples are specified in a project-specific SAP. Samples are immediately stored on wet ice, ice packs, or in a refrigerator and must be processed for analysis within 8 hours. Quality control samples include equipment and dilution blanks and field replicates.

One packet of Colilert® reagent (either 24-hour or Colilert®-18 formula) is poured into each sample bottle and swirled 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 or 18-22 hours depending on the Colilert® reagent. A color change in tray wells from clear to yellow, observed under ambient lighting, indicates the presence of total coliform bacteria. Wells both yellow and fluorescent under UV lighting indicate the presence of *E. coli*.

Counts of small and large yellow and fluorescent wells are used in conjunction with the IDEXX MPN table to determine the number of each type of bacteria.

3.0 DEFINITIONS

Aseptic technique: Performed under sterile conditions.

Colilert® Comparator: IDEXX Quanti-Tray® used to assist in distinguishing positive from negative results for Total Coliform and *E. coli.*

Deionized water: Water with the ions removed. Also shortened to DI.

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.

Thalweg: The deepest and fastest part of the channel (most often), containing the most cross-sectional flow.

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 (wavelength of light, shorter than visible light, < 400 nm).

4.0 HEALTH AND SAFETY WARNINGS

Hazardous conditions potentially exist at every waterbody. If unfavorable conditions are present at the time of sampling, it is recommended that the sampling be rescheduled. If hazardous conditions arise during sampling, such as lightning, high winds, rising water, or flash flood warning, personnel should cease sampling and move to a safe location.

Take appropriate precautions when operating equipment and working on, in, or around water, as well as possibly steep and unconsolidated banks, bridges, or edges of ponds/lagoons. Use caution when working in waders as drowning hazards exist. All field crews should follow DWQ health and safety procedures and be equipped with safety equipment such as proper wading gear, personal flotation devices (PFDs), gloves, first aid kits, cellular phone, etc.

When working in Utah and other warm climates, take precautionary steps to avoid heat induced illnesses such as heat stroke or heat exhaustion.

Wear safety glasses and avoid looking directly into the UV light.

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 washing their hands frequently.

Take 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. The Colilert® reagent is not hazardous according to the manufacturer's MSDS but inhaled powder may cause lung irritation.

5.0 CAUTIONS

Use only designated IDEXX trays and bottles. Autofluorescent plasticware or glassware may produce false positives.

Be sure to store samples on ice or in the refrigerator but do not freeze them; freezing can damage bacterial cells.

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

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 native water.

Verify that the incubator temperature is at 35 ± 0.5°C to ensure valid incubation. Be careful when filling the incubator with cold samples to maintain 35°C.

Ensure the incubator is not overfilled to restrict air flow.

6.0 INTERFERENCES

Avoid surface scum and sediment plumes to obtain a representative water sample.

Avoid sampling slack water or back eddies. Collect a representative sample from the thalweg.

Samples must be collected in sterile containers. Do not touch the insides of the bottle or cap; keep hands near the base of the bottle while sampling. It is critical to use aseptic technique to avoid sample contamination.

E. coli concentrations can be affected by water temperature, increased UV radiation, and runoff pulses. If possible, be consistent regarding the sampling time and sun exposure during sample collection at a particular location and record anomalous site conditions on field forms. Immediately place samples in a cooler.

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

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

DWQ personnel performing water sampling must be familiar with sampling techniques, safety procedures, proper handling, and record keeping. Samplers are responsible for attending refresher meetings held each spring/summer to review procedures and techniques. New staff will be trained in the field and lab by DWQ personnel.

Cooperators 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**) and re-demonstrate on a yearly basis or as needed.

8.0 EQUIPMENT AND SUPPLIES

Note: All Colilert® supplies are purchased through IDEXX. Check expiration dates before using.

☐ Copy of this SOP

☐ Site portfolio or copy of project-specific SAP

☐ Field data sheets (**Appendix 3**) or project-specific field sheets

☐ Water-proof pens/pencil and Sharpie

- ☐ Sterile 120-mL polystyrene bottles containing sodium thiosulfate [IDEXX cat# WV120SBST-20 (20 pack) or WV120SBST-200 (200 pack)]
- ☐ Field thermometer (recommended), NIST-Traceable
- ☐ Deionized (DI) water (preferred)
- ☐ Maps/GPS locations
- ☐ Camera
- ☐ Cooler
- ☐ Wet ice or ice packs
- ☐ Safety gear (gloves and/or hand sanitizer)
- ☐ Chest waders with belt or hip boots

For processing samples, the following supplies are also needed:

- ☐ Data sheet, computer or tablet to record results
- ☐ Quanti-Trays®/2000 [IDEXX cat# WQT-2K (100/box)]
- ☐ 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)]
- ☐ Colilert® Comparator [IDEXX cat# WQT2KC]
- ☐ IDEXX Quanti-Tray® Sealer
- ☐ Rubber insert for sealer [IDEXX cat# WQTSRBR-2K]
- ☐ Incubator and power supplies
- ☐ Incubator thermometer, NIST-Traceable [FisherSci Cat# S66278]
- ☐ Handheld 6-watt long wave UV lamp: 110 volts [IDEXX Cat# 98-20724-01]
- ☐ Sharpie and Pen/Pencil
- ☐ MPN Table
- ☐ Autoclavable Biohazard Bags [VWR Cat #14220-086]
- ☐ Elastic closures for biohazard bags [Fisher Scientific #01-815-8]

If performing dilutions:

☐ Sterile 120-ml polystyrene bottles containing sodium thiosulfate [IDEXX cat# WV120SBST-20 (20 pack) or WV120SBST-200 (200 pack)]

☐ Deionized (DI) water (preferred)

☐ Sterile, disposable pipets (1 mL to 25 mL) [FisherSci Cat# 03-395-188]

☐ Pipettor

9.0 PROCEDURE

9.1 Collection

This procedure assumes grab sampling techniques will be used. If samples must be collected from bridges or with the use of a sampling device, and samples must be collected in containers other than the sample bottle, the containers must be sterile. Refer to DWQ's SOP for Collection of Water Chemistry Samples for explanation of grab sampling and other sampling techniques.

9.1.1 Pre-Sampling Preparation

- Determine the total number of samples (including QC samples such as blanks and/or replicates) to be collected for the sampling event. Determine if original Colilert® (24-hr) or Colilert®-18 reagent will be used. This may depend on sample collection time and when the sampler is available to read the results of the analysis. Gather the appropriate supplies (See **Section 8.0** of this SOP).

- Determine the sampling locations. This information should be included in a project-specific SAP.

    - Any sites that are going to be sampled routinely should have a DWQ site ID. See the *E. coli* Monitoring Program Coordinator to obtain a site ID for your sampling location.

- Obtain any necessary permission for site access.

- Before leaving the office, make sure to have necessary field forms either printed or electronic. Ensure the incubator is plugged in and set to 35°C. If samples will be processed at a cooperator's facility, contact the facility prior to sampling (preferably one week's notification).

9.1.2 Reservoir and Lake Collection Procedures

At lakes/reservoirs collect three transect grab samples (triplicates). Triplicate samples are designed to capture the variability of *E. coli* concentrations at the sampling site. Water temperature measurements are recommended, but refer to the SAP to determine if water temperature is required. See **Appendix 4** for an overview of sample collection at lakes.

1) Upon arrival at the lake/reservoir, check the GPS coordinates to locate the predetermined site. If sampling a new site, record GPS coordinates.

2) Label the sample bottles with the site ID, date, and time of collection. The three bottles for the transect samples are labeled with the same time.

3) Gather the field thermometer (recommended) and 3 unopened sample bottles and wade into the reservoir until knee deep.

4) Remove the lid of the sample bottle. Holding the bottle near the base, gently tilt the bottle, careful not to pour out the sodium thiosulfate. Plunge the bottle to a depth of approximately 12-18 inches (or elbow depth) to collect the water sample and fill to the 100 mL line.

Note: Avoid sampling surface scum and bottom sediments.

5) If you fill the bottle above the 100 mL mark, flick your wrist to bring the volume down to the 100 mL mark but not below (volumes below 100 mL may lead to invalid test results).

Note: To avoid contamination, be careful not to touch the inside of the lid or bottle.

6) Replace the lid securely and shake the bottle for a few seconds to mix the sample and sodium thiosulfate. Walk 10 feet in one direction (paralleling the shoreline) to grab the second transect sample; then walk another 10 feet further to grab the third transect sample.

Note: Take extra care when paralleling the shoreline to minimize disturbance of the bottom sediments.

7) Measure water temperature.

8) Return to shore and store the samples in a cooler on wet ice or ice packs.

9) Fill out field form accurately and completely with sampling location, time, date, and water temperature.

10) Record comments about any unusual water, weather or any activity that may influence results (leaking pipes, animals, sewage smells, etc.)

11) If collecting replicates, label a second set of bottles with site ID, date, and time. Replicates have a unique site ID. Wade out to kneed depth to collect triplicate samples in the same transect sampling method. See **Section 11.2**.

12) As needed, while in the field, prepare an *equipment blank* (discussed in **Section 11.1** of this SOP) by pouring 100 mL deionized water into a 120 mL sample bottle. Place the equipment blank in the cooler.

13) Process and incubate samples within 8 hours of collection. For sample processing, see **Section 9.2**.

9.1.3 Rivers/Stream Collection Procedures

Sample collection at rivers/streams will include the collection of only one sample unless specified otherwise in the SAP. Water temperature measurements are recommended, but check the SAP to determine if water temperature is required. See **Appendix 4** for an overview of sample collection at rivers/streams.

1) Upon arrival at the river/stream, check the GPS coordinates to locate the predetermined site. If sampling a new site, record GPS coordinates.

2) Label the 120 mL sample bottle with the site ID, date, and time of collection. If collecting duplicate or triplicate samples, label with the same time.

3) Gather your field thermometer (recommended) and sample bottle/s. Wade out into the thalweg; wait for any stirred up sediment to move downstream with the current. If it is unsafe to enter the thalweg and can be reached from the bank, collect the sample by reaching into the thalweg where the water is well mixed.

Note: Avoid sampling slack water or back eddies to ensure a representative sample from the thalweg.

4) Remove the lid of the 120 mL bottle. Holding the bottle near the base, gently tilt the bottle, careful not to pour out the sodium thiosulfate. Pointing the opening of the bottle upstream, plunge the bottle to a depth below the river surface and fill up the bottle to the 100 mL line.

5) If you fill the bottle above the 100 mL mark, flick your wrist to bring the volume down to the 100 mL mark but not below (volumes below 100 mL may lead to invalid test results).

Note: To avoid contamination, be careful not to touch the inside of the lid or bottle.

6) Replace the lid securely and shake the bottle for a few seconds to mix the sample and sodium thiosulfate. If applicable, collect the duplicate and triplicate at the same site and in the same manner as the first.

7) Measure the water temperature and return to the bank.

8) Store the samples in a cooler on wet ice or ice packs.

9) Fill out the field sheet accurately and completely with location, time, date, and water temperature.

10) Record comments about any unusual water, weather or any activity that may influence results (leaking pipes, animals, sewage smells, etc.)

11) If collecting replicates, label a second set of bottles with site ID, date, and time. Replicates have a unique site ID. Collect in the same manner as the initial sample. See **Section 11.2**.

12) As needed, while in the field, prepare an *equipment blank* (discussed in **Section 11.1** of this SOP) by pouring 100 mL deionized water into a 120 mL sample bottle. Place the equipment blank in the cooler.

13) Samples must be processed and in the incubator within 8 hours of collection.

9.2 Sample Analysis

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[1]*. 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.*

9.2.1 Preliminary Procedures

1) Make sure the incubator is turned on and set to 35°C.
2) Immediately prior to processing the samples, verify that the temperature of the incubator is 35 ± 0.5°C using the internal thermometer. (If using a portable incubator, check frequently to ensure constant temperature. Use in a location not likely to experience fluctuations in ambient temperature.) It is important that the incubator maintains a consistent temperature of 35 ± 0.5°C.
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.

Note: If concerned about maintaining the 35°C once the incubator is filled with cold trays, warm the samples in a 35°C water-bath (warm tap water) for 15-30 minutes before proceeding. ***This step is critical if there are already samples in the incubator to help maintain a constant temperature.***

9.2.2 Sample Preparation

1) Wash your hands and put on clean, disposable gloves.
2) Label the trays with the sample location and time of collection. Record incubation start date, time, and sampler initials on a data sheet (**Appendix 3**) attached to the incubator for easy reference. Make sure the information on each sample bottle correctly matches the labeled tray.
3) One Colilert® reagent snap packet is required per 100 mL water sample. 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, careful not to touch the rim of the bottle. Make sure to face the snap pack away from you before opening. Swirl the sample bottle to dissolve the powder, trying not to cause excessive foaming. Make sure the reagent is completely dissolved before continuing to the next step.

9.2.3 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.

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

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.

9.2.4 Incubation

1) Place sealed trays in the incubator and incubate at 35 ± 0.5°C for 24-28 hours or if using Colilert®-18, incubate trays for 18-22 hours.
2) Avoid over-stacking trays by leaving airspace above and around stacks. Place trays face down to avoid potential leakage.
3) Fill out the data sheet completely (**Appendix 3**). Record the starting date, time and temperature of the incubator. Also, record expiration dates of Colilert®, bottles and trays.
4) When incubation is complete remove trays from the incubator for interpretation. Be sure to record the ending date, time, and incubator temperature on the data sheet.

9.2.5 Interpretation

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.

When counting wells for total coliform and *E. coli*, count large and small wells independently (**maximum: 49 large wells, 48 small wells**).

1) Count the number of large and small wells that are yellow under normal lighting. The yellow color is indicative of the presence of total coliforms. Record these counts on the data sheet or submission form. Mark the yellow cells with a sharpie prior to reading under the UV light.
2) 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 for Colilert® or 22 if using Colilert®-18). 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.
3) 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 UV light. If the well is fluorescent and yellow, count and record the number of fluorescent wells. Wells are only considered positive for E. coli if they are both yellow and fluorescent.
4) The equipment blank should remain colorless and negative for fluorescence.
5) 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.

b) In general, an empty well is considered negative.[2] However, if all wells are positive, count as positive. Having up to 2 empty wells will not result in a statistically significant difference in measured total coliform/*E. coli* concentrations.

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

9.2.6 Dilutions

There are situations when the numerical value from the 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. This will be outlined in the SAP. 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. If dilutions are predetermined by the SAP, be sure to use the designated DWQ site ID to report results.

1) If dilutions are needed take two samples at the sampling site. Analyze one sample undiluted and analyze the second sample at a 1:10 dilution.
2) 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 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 data sheet accordingly.
3) Prepare a dilution blank by analyzing 100 mL of the deionized water used for making dilutions as a regular sample.
4) 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).

9.2.7 Data Analysis and Calculations

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

1) Use the MPN tables (Appendix 1).
2) Calculated automatically via DWQ generated electronic form.
3) MPN Generator provided by IDEXX (https://www.idexx.com/en/water/resources/mpn-generator/)

Be sure when reporting data, the MPN value is correct.

Note: 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 water, call DWQ to discuss, etc.).

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

9.3 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.

10.0 DATA AND RECORDS MANAGEMENT

Sample bottles should be labeled with the sampling location, date and time of collection. Complete field data sheets in the field before moving to the next sampling location. Samplers should record on field data sheets any site conditions that may lead to an unrepresentative sample and should take site photographs to record these observations. Samplers should also be observant of any potential sources of fecal pollution at the sampling location or in the surrounding area, comment on these observations on the field data sheet or in field notes, and notify the project manager upon returning to the office.

If transposing data from a data sheet to a submission file, cross-check data to ensure correctness. Retain any data sheets for 3 years.

Data collected for recreational health or sites highlighted as high recreation areas should be submitted as soon as possible. If the data is not prioritized for recreation, it should be submitted to the *E. coli* Monitoring Program Coordinator monthly or quarterly as specified in the SAP. Data should include sample collection date, time, site ID, number of "positive" large and small wells for total coliform and *E. coli*, calculated MPN, collector's initials, dilution factor (if applicable), incubation time, date and temperature details and expiration dates of supplies.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

QA/QC procedures are needed in the *E. coli* Monitoring Program to ensure the validity of analytical data and to collect data of the quality and quantity required to make defensible assessments. QC samples include equipment and dilution blanks and replicate samples.

Only use the unused, sealed sterile 120 mL sample bottles with 10 mg sodium thiosulfate provided for sample collection. Use aseptic technique throughout sample collection procedures.

Each DWQ analyst must read this SOP annually and date and sign the form kept at DWQ. Each new DWQ analyst should complete an initial Demonstration of Capability (DOC) to detect and enumerate *E. coli* by the approved Colilert® method. These forms will be kept in the tracking file "[YEAR] DWQ SOP Acknowledgement and Training Form."

DWQ cooperators will follow the above process, but their training and DOC paperwork will be tracked by the Cooperator Program Coordinator, Ellen Bailey.

11.1 Equipment and Dilution Blank Samples

For the purposes of *E. coli* monitoring, equipment and dilution blank samples are used to determine if contamination is present during the collection, handling, storage, transport, and processing of samples. An equipment blank will be prepared daily or monthly during the sampling run to ensure one blank for every 10 samples or 10%. Each project will have a designated blank site ID.

Create the equipment blank in the field by filling a 120 mL bottle with deionized water, replacing the lid, and shaking the sample for a few seconds to mix. Label the bottle with the "BLANK" site ID, time and process the equipment blank as a normal sample.

When performing a dilution, create a dilution blank in the lab using the same deionized water source as used in the dilution. Label dilution blank with appropriate DWQ site ID as specified in the SAP and process as a normal sample.

11.2 QA/QC Field Replicate Samples

Replicate *E. coli* samples are used to quantify the variability in sample collection as well as in sample handling and analysis. One replicate sample should be collected for every 10 sampling locations; the goal is to have replicates account for 10% of the total samples collected during the recreation season. Refer to the SAP or contact the *E. coli* Monitoring Program Coordinator to determine if a replicate is required.

For **lakes/reservoirs**, perform a replicate sample by collecting a second set of transect samples after completing the initial sample. Label these bottles as with the replicate site ID and assign a different collection time from the initial sample. Collect replicate samples in the same manner as the initial sample using the transect sampling method.

For **rivers/streams**, perform a replicate sample by collecting a second bottle (or a second set of 2 or 3 bottles if collecting duplicate/triplicate samples). Label these bottles as with the replicate site ID and assign a different collection time from the initial sample. Collect replicate samples in the same manner as the initial sample.

11.3 Additional QA/QC Considerations

- Record the temperature of the incubator using the internal thermometer when the samples are initially placed inside the incubator and when the samples are removed.

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

- Verify proper sample collection, handling, and analysis by testing equipment and dilution blanks and field replicates. Prepare equipment blanks with deionized water daily or monthly at a frequency of 10%. Test for replicates at a frequency of 10%. If you do not have a good source for deionized water, coordinate with the *E. coli* Monitoring Program Coordinator to procure some.

- Use the same deionized water source for equipment blanks and dilution blanks (to capture potential for false positives during dilution preparation).

- Use Colilert® reagent, bottles and trays within the expiration date. Store reagent in a cool, dark place.

- Store Comparator in a dark place and replace when expired.

- Check incubation and field thermometers annually against NIST-traceable thermometer and replace them if the difference is greater than 1°C. DWQ has an NIST-traceable thermometer.

12.0 REFERENCES

DWQ's Integrated Report
ir.utah.gov

IDEXX Product Inserts

Standard Methods for the Examination of Water and Wastewater. 23rd edition. Edited by Clesceri *et a*l.

U.S. Environmental Protection Agency, 2014e, National beach guidance and
required performance criteria for grants: EPA 823-B-14-001
User Manual, Quanti-Tray® Sealer. IDEXX Laboratories, Inc., Westbrook, Maine.

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

Related DWQ SOPs:

Standard Operating Procedure for Collection of Water Chemistry Samples

13.0 APPENDICES

APPENDIX 1 - SOP ACKNOWLEDGMENT AND TRAINING FORM

SOP Acknowledgement Form

This SOP must be read and acknowledged annually, and this form must be kept on file at DWQ.

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."

Date	Printed Name	Signature

SOP Training Form

Training is required for all new samplers and Refreshers are required annually.

Trainee: Sign below to acknowledge that training on this SOP was received, understood, and all questions/concerns were addressed by the trainer.

Trainer: Sign below to acknowledge that training on this SOP was completed for the individual listed and that trainee is competent to perform the procedures described within.

Date	Trainee Printed Name	Trainee Signature	Trainer Printed Name	Trainer Signature
Management Approval				
Printed Name:				
Signature:				
Date:				

APPENDIX 2 – DOC FORM

Demonstration of Capability (DOC) Form for *E. coli* Testing

Analyst Name (print):	
Analyst Signature:	
Date of DOC:	
Analytical Method(s):	IDEXX Colilert with Quanti-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 (print):	
Trainer Signature:	
Mgmt Approval Signature:	

APPENDIX 3 – DATA SHEET

UTAH DEPARTMENT of ENVIRONMENTAL QUALITY
WATER QUALITY

Sampling Information: | **Method: IDEXX Colilert** | **Sample Materials:**

Trip ID: _____
Project Code: _____
Agency Code: _____
Personnel: _____

☐ Colilert (24 hr)
☐ Colilert (18hr)

Colilert Exp Date: _____
Tray Exp Date: _____
Bottle Exp Date: _____

Incubation Start

Date IN: _____
Time IN: _____
Temp IN (°C): _____

Incubation End

Date OUT: _____
Time OUT: _____
Temp OUT (°C): _____

Data Management (DWQ only)

Data Formatted? _____
Data Sheet Scanned? _____

MLID	Description	Rep #	Sample Date	Sample Time	Dilution	Total Coliform		E. coli		E. coli MPN	Water Temp °C	Comment
						# LRG	# SML	# LRG	# SML			

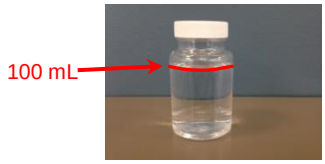
APPENDIX 4 - QUICK SAMPLING INSTRUCTIONS

Sample Collection Instructions

Materials – 120mL bottles, Sharpie, cooler with ice, waders, thermometer, site locations, data sheet, pencil, De-ionized (DI) Water

- Lakes/reservoirs - Wade out to knee depth
    - Collect samples at 12-18in deep (about elbow depth)
    - Collect three transect samples 10 feet apart parallel to shore (see image below)
    - Avoid collecting surface scum
- Rivers/streams - Collect in the main flow
    - Collect just below the surface
    - Avoid eddies and backwater

1) Label bottles at each site - Time, site name
2) Fill bottle to the 100mL line (better a little too much than not enough).
    - Careful not to spill powder (sodium thiosulfate) in the bottle when filling the bottle.
    - Flick sample to remove excess water
3) Collect water temperature
4) Record site and time on the data sheet.
5) Record water temp in the comments on data sheet
6) Place samples on ice
7) As needed, create equipment blank in the field, filling a bottle with 100mL of deionized water.
8) Process in the lab within 8 hours



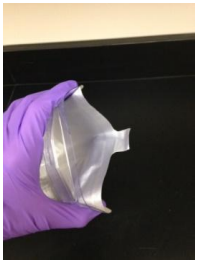
Lake transect sampling instructions:
Walk to knee depth – collect first sample, walk 10 feet parallel to shore to collect second sample, walk another 10 feet parallel to shore to collect third sample.

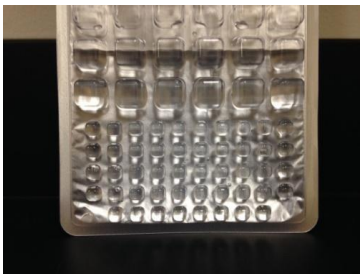
Sample Analysis and Quantification Quick Instructions

Materials – Collected samples, gloves, 18 or 24 hr Colilert, Quanti-tray 2000, data sheet, Sealer and rubber insert, Incubator, Sharpie, pencil/pen, MPN table

1) Before going out to collect samples, turn on the incubator. Make sure incubator is at 35±0.5°C.
2) Turn on Sealer, takes about 5 minutes – light will turn green when ready
3) Put on gloves

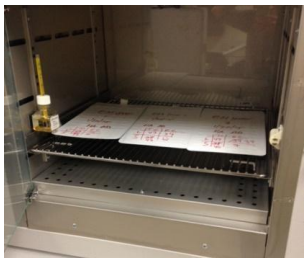
4) Pour Colilert into each sample – swirl to allow powder to dissolve.
5) Label the site and collection time on the trays with Sharpie
6) Once Colilert is dissolved, pour sample into tray and tap tray to dislodge air bubbles





7) Place tray on rubber insert and feed through the sealer making sure the open end is facing away from the sealer. Tray will feed out the back.

8) Distribute trays evenly in the incubator



9) Fill out sample data sheet completely.

E. coli Sample Summary Sheet

Sampling Information:									
Agency:					Trip Id:				
Project Code:					Method:	☐ 24 hr	☐ 18 hr		
INCUBATED			**READ**			**Supplies**			
Date IN:			Date OUT:			Bottle Exp Date:			
Time IN:			Time OUT:			Tray Exp Date:			
Temp IN (°C):			Temp OUT (°C):			Reagent Exp Date:			
MLID	**Date Sampled**	**Time Sampled**	**Total Coliform**		**E. coli**		**Dilution**	**E. coli MPN**	**Comment**
			# LRG	**# SML**	**# LRG**	**# SML**			

10) Incubate for:
- 24 hour Colilert: 24 – 28 hours
- 18 hour Colilert: 18 – 22 hours

Interpretation:

11) Count number of yellow wells (Total Coliform) – count large and small wells individually
12) Count number of both yellow AND florescent wells (E. coli) – count large and small wells individually

Note: If unsure on the color, use a 'Comparator tray' to help distinguish positive or negative or incubate for up to 4 more hours, not to exceed the max incubation time. If color intensifies, well is positive.

13) Empty wells – If one or two empty wells are present, count those wells as a negative, unless all wells are positive in which case they will count as a positive. Three or more empty wells and the sample is invalid.
14) Record values on data sheet or data form provided by the Program Coordinator.
15) Calculate MPN (using the MPN Table, or DWQ Excel file)

Reporting:

16) For recreation monitoring, if sample result value is >WQS or BAV (water quality standard or Beach Action Value),
    a. Contact **Ellen Bailey** (ellenbailey@utah.gov, 801-536-4339). You may be asked to sample again if possible.
17) Review and submit data.