

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

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	Put existing SOP into the new format. Added 18-hr Colilert test option. Added a footnote explaining "duplicate" QC sample terminology. Began document control/revision tracking.
6/27/13	1.1	<ol style="list-style-type: none"> 1) Added the option of watching the training video 2) Added supplies for performing dilutions to equipment list 3) Added creation of MLID for routinely sampled sites 4) Reduced time for shaking sample and added field sheet 5) Changed duplicate frequency to 10% for entire rec season and asked samplers to assign a different time to duplicate sample 	<ol style="list-style-type: none"> 1) 7.0 2) 8.0 3) 9.1 4) 9.2 and 9.3 5) 11.2 	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 water sample collection from Utah's lakes and rivers/streams for *Escherichia coli* (*E. coli*) analysis. This SOP covers sample collection only; it does not cover sample analysis. Processing and analysis of *E. coli* samples is discussed in DWQ's SOP for *Escherichia coli* (*E. coli*) and Total Coliform Quantification using the IDEXX Quanti-Tray/2000 System. This SOP applies to all personnel collecting *E. coli* samples including DWQ monitors, non-DWQ cooperators, and volunteer monitors.

E. coli concentrations are used in water quality assessments 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:

Sandy Wingert
Watershed Protection Section
Utah Department of Environmental Quality
PO BOX 144870
Salt Lake City, UT 84114-4870
(801) 536-4338
swingert@utah.gov

2.0 SUMMARY OF METHOD

Water samples (minimum 100-mL volume) are collected in sterile 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. Routine/baseline river/stream samples are collected in one bottle; reservoir/lake and targeted "hot spot" river/stream samples are collected in triplicate (3 bottles). 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 field blanks and duplicates.

3.0 DEFINITIONS

aseptic technique:	performed under sterile conditions
<i>E. coli</i> :	<i>Escherichia coli</i>
ml:	milliliters
QA/QC:	quality assurance and quality control

SAP: sampling and analysis plan

UV: ultra violet

4.0 HEALTH AND SAFETY WARNINGS

Field personnel should be aware that 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.

Field personnel should 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. All field crews should follow EPA, OSHA, and specific 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.

Avoid exposure to pathogens; wear gloves or be sure to wash hands or use hand sanitizer after sampling water potentially contaminated with fecal pollution.

5.0 CAUTIONS

Samples must be collected in sterile containers.

Avoid sample contamination; do not touch the inside, lip, or cap of the sampling container.

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

6.0 INTERFERENCES

Attempt to collect the water samples from a minimum depth of 6 inches in rivers and 12 inches in lakes or reservoirs. Avoid surface scum and sediment plumes in order to obtain a representative water sample.

E. coli concentrations are affected by water temperature and UV radiation. Levels at beaches are higher in the morning than afternoon and higher in the shaded areas than in areas exposed to full sun. If possible, be consistent regarding the sampling time and sun exposure during sample collection at a particular location. Immediately place samples in a cooler in the dark; do not let samples sit out in sunlight.

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.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

Samplers 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.

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 will be trained in the field by experienced personnel.

8.0 EQUIPMENT AND SUPPLIES

- _____ Copy of this SOP
- _____ Site portfolio or copy of project-specific SAP
- _____ Field data sheets (**Appendix 2**) 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

If processing samples, the following supplies are also needed:

- _____ Copy of DWQ's SOP for *Escherichia coli* (*E. coli*) and Total Coliform Quantification using the IDEXX Quanti-Tray/2000 System
- _____ Incubation data sheets (bench sheets)
- _____ Quanti-Trays/2000 [IDEXX cat# WQT-2K (100 trays)]
- _____ 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)]
- _____ Thermometer
- _____ Sealer (IDEXX cat# WQTS2X-115) and Rubber insert for sealer (IDEXX cat# WQTSRBR-2K)
- _____ Incubator and power supplies
- _____ UV lamp
- _____ Sharpie and Pen/Pencil
- _____ Deionized/distilled water (preferably sterile)
- _____ MPN Calculator or MPN Table
- _____ 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

9.0 PROCEDURE

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 Pre-Sampling Preparation

- Determine the total number of samples (including QC samples such as blanks and/or duplicates) 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.
 - For sites that have previously been sampled for *E. coli*, use GPS coordinates to locate and confirm the sampling location.
 - For new sites, the sampler should determine the sampling location and record its coordinates. The preferred sampling location (in order of

priority) will be designated beaches, lakeside camping areas, and non-designated beaches.

- For rivers, collect samples in the main flow at the designated monitoring site.
 - Any sites that are going to be sampled routinely should have a Monitoring Location ID (MLID, a site code formerly referred to as a STORET by DWQ). See the Bacteriological Program Coordinator to obtain a MLID for your sampling location.
- Obtain any necessary permission for site access.
 - Before leaving the office, make sure the field and bench sheets are printed. If using a portable incubator, plug it in to ensure that it has power and comes up to temperature. If samples will be processed at a cooperator's facility, contact the facility prior to sampling (preferably one week's notification).

9.2 Reservoir and Lake Collection Procedures

At lakes/reservoirs collect three individual grab samples (triplicates). Triplicate samples are designed to capture the variability of *E. coli* concentrations at the sampling site. See **Table 1** 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 no coordinates are given, take GPS coordinates along the shoreline at the first sampling point and record them.
- 2) Label the sample bottles with the sampling location, replicate number, date and time of collection.
- 3) Before collecting lake/reservoir samples, prepare a *field blank* (discussed in **Section 11.0** of this SOP) by pouring 100 ml distilled/deionized water into a 120 ml sample bottle. Place the field blank in the cooler. Perform one field blank daily at the first sampling location and handle and process as a regular sample.
- 4) Gather the field thermometer and 3 unopened sample bottles and wade into the reservoir until knee deep.
- 5) Measure and record the water temperature.
- 6) Remove the lid of the sample bottle and invert the bottle to a depth of approximately 12-18 inches to collect the water sample while avoiding surface scum and bottom sediments. Be careful not to accidentally pour out the sodium thiosulfate when inverting the bottle. Fill up the bottle to the 100 ml line. 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 difficult-to-

interpret or invalid test results). To avoid contamination, be careful not to touch the inside of the lid or bottle.

- 7) 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 replicate; then walk another 10 feet further to grab the third replicate sample. Take extra care when paralleling the shoreline to minimize disturbance of the bottom sediments (i.e. do not sample the kicked up sediment plume).
- 8) Store the samples in a cooler on wet ice or ice packs.
- 9) Fill out field sheet accurately and completely.
- 10) Samples must be processed and in the incubator within 8 hours of collection. For sample processing, refer to DWQ's SOP for *Escherichia coli* (*E. coli*) and Total Coliform Quantification using the IDEXX Quanti-Tray/2000 System.

Table 1. Overview of E. coli sampling at reservoirs/lakes.

1. Check GPS coordinates to confirm sampling site.
2. Label 120 ml bottles with sampling location, replicate number, time, & date of collection.
3. Prepare the field blank using distilled/deionized water (perform one field daily at the first sampling location).
4. Gather thermometer and 3 sterile sample bottles and wade into reservoir till knee deep.
5. Record water temperature. Gently invert and fill up first replicate at a depth of 12-18 inches. Replace lid securely and shake bottle to mix.
6. Walk 10 feet in one direction paralleling shoreline and fill up the second replicate. Repeat again thus collecting 3 samples (triplicates).
7. Take note to fill bottles as accurately to the 100 ml mark as possible.
8. Fill out field sheet accurately and completely.
9. Store samples on wet ice/ice packs. Process samples within 8 hours of collection. See <i>E. coli</i> Quantification SOP for processing.

9.3 Stream/River Collection Procedures

Routine/first-time sample collection at rivers/streams will include the collection of only one sample. If a river/stream is designated a “hot spot” for Tier 2 targeted monitoring (see DWQ’s Integrated Report), sample collection will involve triplicate samples. For questions regarding whether your site is a “hot spot”, contact the Bacteriological Program Coordinator for instructions. See **Table 2** for an overview of sample collection at rivers/streams.

- 1) Upon arrival at the river, check the GPS coordinates to locate the predetermined site. If no coordinates are given, take GPS coordinates along the bank and record them.

- 2) Label the 120 ml sample bottle with the sampling location, replicate number (if applicable), date, and time of collection.
- 3) Before collecting lake/reservoir samples, prepare a *field blank* (discussed in **Section 11.0** of this SOP) by pouring 100 ml distilled/deionized water into a 120 ml sample bottle. Place the field blank in the cooler. Perform one field blank daily at the first sampling location and handle and process as a regular sample.
- 4) Wade out into the stream/river's main flow (thalweg) and measure and record the temperature.
- 5) Go back to the bank and gather an unused sample bottle. Wade back into the water to the same location; wait for any stirred up sediment to move downstream with the current.
- 6) Remove the lid of the 120 ml bottle and gently invert the bottle to a depth just below the river surface, filling up the bottle to the 100 ml mark. Avoid surface scum and the stream bottom. Be careful not to accidentally pour out the sodium thiosulfate when inverting the bottle. Fill up the bottle to the 100 ml line. 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 difficult-to-interpret or invalid test results). To avoid contamination, be careful not to touch the inside of the lid or bottle.
- 7) Replace the lid securely and shake the bottle for a few seconds to mix the sample and sodium thiosulfate. If applicable, collect the second and third replicate at the same site and in the same manner as the first.
- 8) It is very important not to sample slack water or back eddies due to variability in *E. coli* densities (bias). Contact the Bacteriological Program Coordinator for recommendations for sampling equipment or adjustment of sampling location if you are routinely unable to sample from the stream thalweg or a well-mixed zone at a particular sampling location.
- 9) Store the samples in a cooler on wet ice or ice packs.
- 10) Fill out the field sheet accurately and completely.
- 11) Samples must be processed and in the incubator within 8 hours of collection.

Note: For sample processing, refer to DWQ's SOP for *Escherichia coli* (*E. coli*) and Total Coliform Quantification using the IDEXX Quanti-Tray/2000 System.

Table 2. Overview of *E. coli* sampling at rivers/streams.

1. Check GPS coordinates to confirm the sampling site.
2. Label 120 ml bottles with sampling location, replicate number, time, & date of collection.
3. Prepare the field blank using distilled/deionized water (perform one field daily at the first sampling location).
4. Wade out to main flow (thalweg) and record the temperature.
5. Wade back to same location with the sterile 120 ml bottle containing 10mg sodium thiosulfate (or 3 bottles if collecting triplicate samples).
6. Fill up sterile bottle(s) just below the water surface, replace lid and shake for 30 seconds. Take note to fill as accurately to the 100 ml mark as possible.
7. Fill out field sheet accurately and completely.
8. Store samples on wet ice. Process samples within 8 hours of collection. See <i>E. coli</i> Quantification SOP for processing.

10.0 DATA AND RECORDS MANAGEMENT

Sample bottles should be labeled with the sampling location, replicate number, and date and time of collection. Complete field data sheets in the field before moving to the next sampling location. Make sure that field data sheets correspond to the sample bottle labels. Send DWQ scanned PDF's of the field sheets or photocopies at the end of each recreational season (or if preferred, monthly with the data). Retain the original field sheets in your records. 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.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

QA/QC procedures are needed in the Bacteriological 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 field blank and replicate samples.

Only use the unused, sealed sterile 120 ml sample bottles provided for sample collection. Use aseptic technique throughout sample collection procedures.

11.1 Field Blank Samples

For the purposes of *E. coli* monitoring, field blank samples are used to determine if contamination is present during the collection, handling, storage, transport, and processing of samples. A field blank will be prepared at the first sampling location at the beginning of each sampling day, before collection of lake/stream samples. Create

the field blank by filling a 120 ml bottle containing 10 mg sodium thiosulfate with distilled/deionized water, replacing the lid, and shaking the sample for a few seconds to mix. Label the bottle as “BLANK” and process the field blank as a normal sample.

11.2 Field Duplicate Samples (Replicate Samples)²

Duplicate *E. coli* samples are used to quantify the variability in sample collection as well as sample handling and analysis. One duplicate sample should be collected for every 10 sampling locations; the goal is to have duplicates account for 10% of the total samples collected during the recreation season.

For lakes/reservoirs, perform a duplicate sample by collecting a second set of triplicate samples immediately following collection of and in the same manner as the normal samples. If possible, wade out with all 6 bottles and thereby disturbing the beach sediments only once. Label these bottles as “DUP” + replicate number and process as normal samples. Assign the duplicate sample a different collection time from the regular sample.

For rivers/streams, collect a second bottle (or a second set of 3 bottles if collecting at a “hot spot”) immediately following collection of and in the same manner as the normal sample. If possible, wade out with all the bottles needed (2 bottles total for routine or 6 bottles total for a “hot spot”). Label these bottles as “DUP” + replicate number (if sampling a “hot spot”) and process as normal samples. Assign the duplicate sample a different collection time from the regular sample.

² True duplicates are not collected for the *E. coli* Program because they would require one volume of water being collected in a large sampling container and poured equally into sterile 120 ml sample bottles. This would require sterilization and decontamination of sampling equipment. These QC samples are actually replicate samples. However, the term “duplicate” is used to avoid confusion with the three replicates collected at lake or “hot spot” sampling locations.

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)

Related DWQ SOPs:

Standard Operating Procedure for *Escherichia coli* (*E. coli*) and Total Coliform Quantification using the IDEXX Quanti-Tray/2000 System

Standard Operating Procedure for Collection of Water Chemistry Samples

13.0 APPENDICES

Appendix 1 - SOP Acknowledgment and Training Form

SOP Acknowledgement and Training 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

SOP Acknowledgement and Training Form (continued)

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 of Training	Trainee Printed Name	Trainee Signature	Trainer Printed Name	Trainer Signature

Management Approval

Printed Name:

Signature:

Date:

Appendix 2 – Field Data Sheet

***E. coli* Collection Field Data Sheet**

Sampler(s): _____

Trip Description: _____

Sample Location	GPS Coordinates	Date	Time	Water Temp (°C)	Weather	Observations (livestock or waterfowl near/in stream, recent rain, turbid water, etc.)	Photos Taken?