

Standard Operating Procedures – Inland Water Sample Collection for Microbial Samples

SCOPE

This document is a standard operating procedure (SOP) for inland microbiological sample collection, prepared by the Surface Water Ambient Monitoring Program (SWAMP). This SOP is appropriate for the collection of fresh, estuarine, and marine water samples for analysis of fecal indicator bacteria (FIB), which may include *Escherichia coli* (*E. coli*), fecal coliform, and enterococcus. In addition, this SOP is appropriate for collecting samples for analysis of microbial source tracking (MST) markers and microbial pathogens. Laboratory-based MST methods are used to link fecal contamination in waterbodies to the feces from specific animal hosts (e.g. human, cow, bird).

CAUTIONS AND INTERFERENCES

Cautions are defined as activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure. Interferences are defined as any component of the process that may interfere with the accuracy of the final product. Cautions and interferences for the collection of microbial samples include:

- Sample containers and equipment must be handled carefully in order to prevent contamination or damage.
- Fecal indicator bacteria samples have short ‘holding times’ or the timeframe beginning with collection in the field to processing by the laboratory. To meet the short holding times, close coordination between field crews and the laboratory is required.
- Collect samples for microbiological examination in clean and sterile containers made of either: non-reactive borosilicate glass or plastic containers. If using Whirlpaks, use appropriate aseptic technique to avoid contamination by the sampler. Where legal action may be involved, consider the use of tamper closures.
- Leave ample air space in the sampling container to facilitate mixing before sample analysis. Some bottles and WhirlPaks have markings with the desired volume; otherwise, bottles should not be filled above the shoulder.
- Samples must be treated with proper preservation to ensure the sample will be useable by the laboratory.

PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

All personnel conducting this procedure must be familiar with health and safety protocols before visiting a site. Personnel should also possess a demonstrated proficiency in the use of all instruments to be used. Personnel are responsible for the implementation of the procedures outlined in this SOP and to ensure that the data generated meets the standards and objectives of the monitoring project. The project or field crew leader should conduct periodic reviews of field personnel to ensure that technical personnel are following procedures in accordance with this SOP ([Quality Systems Assessment for Citizen Monitors Conducting Water Quality Monitoring Field Activities, associated audit forms](#)).

EQUIPMENT AND SUPPLIES

When selecting equipment consider your project needs and study. Be sure that any materials being used are in accordance with the lab’s analytical method. If necessary, contact the testing lab prior to sampling to verify their requirements. The following is a general list of equipment for the field:

- | | |
|---|---|
| <input type="checkbox"/> Copy of this SOP | <input type="checkbox"/> Disposable, powder-free gloves |
| <input type="checkbox"/> Backpack | <input type="checkbox"/> Chain-of-Custody (COC) |
| | <input type="checkbox"/> Field data sheets |

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- Clipboard
- Camera with extra batteries (optional)
- Sample container labels
- Ball point pen/pencil
- Permanent marker
- Sample containers (refer to Table 2)
- Ice chests (may need extra for shipping)
- Ice/Ice packs (will need extra for shipping)
- Waders (optional)
- GPS unit (if available)
- Field safety manual
- First aid kit
- Field watch
- Large resealable plastic bag labeled for Trash
- Sampling pole (optional)
- Large resealable plastic bags (to protect samples from melting ice water)
- Hand sanitizer or wipes for areas without handwashing facilities.

PROCEDURE

Pre-Field Visit Preparation

When preparing field materials, go through equipment and supply checklist and ensure all equipment is packed.

1. Ensure sample containers are sterile and sampling gloves are stored to prevent contamination.
2. Prepare sample containers for necessary quality control samples, including field and laboratory replicates. Refer to Table 1 below.

Table 1. Quality Control Requirements - Field Sampling

FIB Analysis	Frequency	Criteria
Field Blank	One sample per field day	Negative Response
Trip Blank	As determine by QA Officer or QA Leadperson	Negative Response
Lab Duplicate ²	One sample per ten samples or per analytical batch, whichever is more frequent	Calculate Rlog per Standard Methods 9020 Section 8.b
PCR-based MST Marker & Pathogen Analysis	Frequency	Criteria
Field Blank	Per field day	No detection ¹
Filter Blank	Per lot/pack	No detection ¹

¹Or detection at least three cycle threshold (C_T) units above the sample C_T .

² Collect additional sample volume (100 mL) for the lab to analyze the sample as a laboratory split. To collect sufficient volume for the duplicate, use sterile container to collect twice the normal sample volume (e.g. minimum 250ml bottle to ensure sufficient capacity).

Site Selection

Collection of water samples should be conducted prior to or upstream from any other sampling activities that could disturb stream sediments and impact water quality (i.e., the collection of flow, sediment, or aquatic invertebrate samples). While wading to the sampling location, be very careful not to disturb bottom sediments. If sampling in an area

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with muddy substrate, consider using a sampling pole. If a sampling pole is not available, wait until disturbed sediments clear and then proceed with care.

Streams and Rivers

When sampling a stream or river, select a sampling location in moving water (ex. thalweg/glide/pool) at the upper end of the stream reach to be sampled and avoid areas where debris will enter sampling container. Flow rate should be moderate, and creek depth sufficient to submerge the sample container at least 3 cm below the water surface without touching or disturbing bottom sediments.

Swimming Beaches and Lakes

If sampling a large beach area, consider collecting multiple samples along the beach in order to document the range of conditions at that beach. Samples should be collected in approximately 2.5 feet of water.

Sample Collection

1. Select a work area nearby that is as flat as possible and with minimal vegetation. Remove sampling equipment and supplies from the backpack, minimizing contact with soil, vegetation, etc.
2. Label the sample containers including field station codes, sample date, and time. Identify field and/or lab duplicate samples on the label, the COC, and on the field data sheet.
3. Fill out the field data sheet completely, including any unusual sample conditions and to document the sample time, GPS coordinates (if available), and any pictures taken.
4. Use fresh/new gloves for each site. Gloves should be kept clean during transport to the field. They should be fresh/new and only put on directly before opening the bottle for sampling. Take care not to place gloved fingers inside sampling container and avoid touching the opening.
5. Special care should be used at all times to avoid contamination of the inside of the sample container and cap. It is recommended to store bottles in resealable plastic bags before collecting samples to prevent contamination.
6. Do not rinse the inside of sample container and do not pour water into a microbial sampling container from another non-sterilized container. This sample handling is especially important to remember when using pole samplers; to prevent site-to-site contamination do not use poles with dipper attachment and instead use configuration that allows for attachment of sample container.
7. Collect your sample
 - a. **Select a sampling site based on recommendations above.** If wading, wait for the disturbance in the bottom sediments to be minimal and water relatively undisturbed. Alternatively, the sample container may be attached to a sampling pole.
 - b. Invert the sample container and in one quick motion, plunge the mouth of the sampling container into the water about 6 inches below the surface. Then tip sample container (still submerged) to fill. Take the bottle out of the water. If the bottle is filled above the shoulder then pour off enough excess sample so the sample volume is at or near the shoulder. Immediately close/cap container.
 - c. If the stream is shallow, submerge container below the water surface to a depth that does not disturb bottom sediments and remove cap to fill. As the sample water reaches the desired volume or near the

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shoulder of the container, remove container and securely cap/close container. If the container is filled above the desired level, uncap and pour out excess sample.

8. Collect field blank by pouring a sample of distilled water into a sampling container in the field, preserved and shipped to the laboratory with the field samples. Follow the same handling and preservation techniques as the other samples. A minimum of one field blank per field day should be obtained.
9. Double-check the sample bottle caps are tightly sealed and place bottles into resealable plastic bags and on ice in a cooler immediately after collection. If sample location is more than five minutes from the vehicle, bring a secondary transport cooler with ice and transport samples to vehicle. Upon returning to the vehicle, place all samples into the main cooler.
10. Dispose of gloves after collecting samples. Do not reuse gloves for the next site.

Sample Handling and Preservation

Samples should be cooled to $\leq 10^{\circ}\text{C}$ as soon as possible after sampling. On hot days, replenish gel packs in the ice chest prior to shipping or traveling to the lab to ensure samples remain cool. Note that wet ice, loose or bagged, should not be used for shipping due to possibility of contamination and leaks during shipment.

If using an external laboratory for sample processing, contact the testing lab to confirm arrival time or estimated shipping arrival for sample processing (short holding times). To meet 24 hour holding time for ambient monitoring, samples must often be shipped overnight with a courier. Make a copy of the chain of custody form for your own records and place the original within a plastic bag (to prevent damage) in the shipping box or cooler. If the shipping container must be returned, include pre-paid shipping label or arrange for the laboratory to return the container for an additional fee.

Table 2. Sample Holding Times for Each Analyte.

Analyte	Maximum Holding Time ⁴	Storage Conditions ¹	Preservative
Fecal Coliform (FIB group)	Ambient monitoring ² : 24 hours Compliance monitoring ³ : 8 hours	Cool to $\leq 10^{\circ}\text{C}$;	If samples may contain chlorine, sodium thiosulfate should be pre-added to containers
<i>E. coli</i> (FIB group)	Ambient monitoring: 24 hours Compliance monitoring: 8 hours	Cool to $\leq 10^{\circ}\text{C}$	If samples may contain chlorine, sodium thiosulfate should be pre-added to containers
Enterococcus (FIB group)	Ambient monitoring: 24 hours Compliance monitoring: 8 hours	Cool to $\leq 10^{\circ}\text{C}$	If samples may contain chlorine, sodium thiosulfate should be pre-added to containers
PCR-based MST Marker & Pathogen Analysis	Ambient monitoring: TBD based on literature review Compliance monitoring: ≤ 6 hours, then filter water and freeze filter for up to 1 year	Water: Cool to $\leq 10^{\circ}\text{C}$ Filter: -25°C minimum	none

¹ Storage conditions should comply with Standard methods 9060 A for Collection and 9060 B for Preservation and Storage.

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²Ambient monitoring is for assessment of a water body, but may not meet the strict quality assurance protocol to be used for compliance or enforcement by the Water Board

³Compliance monitoring is to evaluate if a water body meets the water quality evaluation criteria or permit requirements for a pollutant. It must comply with USEPA standard methods.

⁴ Holding Time begins at sampling time and ends at initial lab processing that includes 1 of the following procedures: filtering, extraction, incubation, or analysis. FIB holding times are from Standard Methods - Section 9060 B. Preservation and Storage

Table 3. Containers Required to Meet Sampling Objectives

Analyte	Required Container	For Lab Replicate
Fecal Coliform	125 mL or 250 mL Factory-sealed, Sterile Bottle or WhirlPak	250 mL Factory-sealed, Sterile Bottle or WhirlPak
E.coli	125 mL or 250 mL Factory-sealed, Sterile Bottle or WhirlPak	250 mL Factory-sealed, Sterile Bottle or WhirlPak
Enterococcus	125 mL or 250 mL Factory-sealed, Sterile Bottle or WhirlPak	250 mL Factory-sealed, Sterile Bottle or WhirlPak
Microbial Source Tracking	1L Autoclaved or new sealed Bottle	Sufficient volume so lab can make replicate

Laboratory Communication and Analysis

Communicate with the laboratory at least 24 hours prior to the sampling event to ensure they can meet the hold time, confirm which hold time applies to your project, and provide an estimated time of arrival. Ensure the Chain of Custody is completed to the specifications of the laboratory and that samples arrive with enough time for the lab to process them within the appropriate holding time.

Equipment Cleaning

All sampling equipment used for sample collection, processing, and handling must be cleaned before use. Plan to clean equipment before the first use each day. If sample containers will be reused, ensure they are autoclaved properly before future use. Field equipment should be cleaned with a phosphate-free laboratory detergent (i.e. Liquinox™) and a soft scrub brush or sponge. If you suspect the equipment has been contaminated before use in the field, a disinfectant should also be used. Disinfectants can include a 70% ethanol or isopropyl solution with an expiration date of 180 days. For all chemicals, review the appropriate Safety Data Sheet before use and follow all manufacturer instructions. Note - alcohols are flammable and volatile, consider safe handling practices.

Be sure to properly dispose of any latex gloves and other consumables used during sampling.

REFERENCES

http://www.ecy.wa.gov/programs/eap/qa/docs/ECY_EAP_SOP_BEACHProgramBacteriaSampling_v1_0EAP092.pdf

SWAMP Quality Control and Sample Handling Guidelines for Indicator Bacteria in Fresh Water:

http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/mgo/updated_ind_bact_water.pdf

**SWAMP BACTERIA
FIELD DATA SHEET**

SWAMP (Surface Water Ambient Monitoring Program)
is administered by the State Water Resources Control Board

Sample Date:		Sample Time (<i>first sample</i>):			Station Code: optional					
Waterbody Name:				Sampling Crew/ Email/Phone:						
Sampling Site Name:										
SAMPLING LOCATION										
LOCATION: Bank, thalweg, midchannel, open water, other: _____					STARTING BANK (facing downstream): LB / RB / NA					
SAMPLE LOCATION: Shore, beachline, wade, boat, dock, bridge, other: _____						Datum: NAD83, WGS84				
TYPE OF SAMPLE: Freshwater, Saltwater, Estuarine										
GPS DEVICE:			GPS/DGPS:		Lat (dd.ddddd):		Long (-dd.ddddd):			
Location Description: (<i>include landmarks</i>)										
SAMPLES TAKEN FOR LABORATORY ANALYSIS										
SAMPLE TYPE: Grab / Integrated			COLLECTION DEVICE:		Indiv. bottle w/ gloved hand, Whirlpak, indiv. bottle w/ pole, bucket, other: _____					
Sample ID			Sample Volume	Fecal coliform	E. coli	Enterococcus	qPCR	Other: _____	Other: _____	Other: _____
FIELD MEASUREMENTS (optional)										
WATER TEMP (°C)							PHOTOS:			
AIR TEMP (°C)										
RAINFALL		<24 hours, < 48 hours, <72 hours, > 72 hours since last rain event								
WEATHER CONDITIONS		sunny, mostly sunny, partly sunny, mostly cloudy, cloudy								
SITE ODOR:		None, Sulfides, Sewage, Petroleum, Smoke, Other								
OTHER PRESENCE		Vascular, Nonvascular, OilySheen, Foam, Trash, Other								
WATERCLARITY:		Clear (see bottom), Cloudy (> 4" vic), Murky (< 4" vis)								
WATERODOR:		None, Sulfides, Sewage, Petroleum, Mixed, Other								
WATERCOLOR:		Colorless, Green, Yellow, Brown							ADDITIONAL NOTES:	
ANIMAL PRESENCE:		None, Birds (#:____), Domestic (#:____), Wildlife (#:____), Not Recorded								
BATHERS:		# of people in water: ____ ; # of people at beach: ____ ; # of people out of water: ____								

**SWAMP BACTERIA
CHAIN OF CUSTODY (COC) FORM**

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Date: _____
Sampling Organization: _____
Project: _____
Field Crew: _____

Sample Location/Water Body Name	Sample ID	Collection Date	Collection Time	Recommended Dilution	Notes

Samples Relinquished by:	Samples Received by:
Name (print and sign) _____	Name (print and sign) _____
Date and Time _____	Date and Time _____