

STANDARD OPERATING PROCEDURE
DIRECT GRAB SAMPLE COLLECTION WITH SAMPLE BOTTLE
FOR FECAL COLIFORM ANALYSIS

* Adapted from City of Portland (Oregon), Environmental Protection Agency, and Washington State Department of Ecology SOPs

1.0 Purpose and Scope

This document is the Whatcom County Public Works- Natural Resources Standard Operating Procedure (SOP) for collecting grab samples directly into sample bottles or containers for the purposes of surface water and/or stormwater sampling and analysis.

2.0 Scope and Applicability

The procedures described in this document pertain to the proper collection of grab samples for laboratory analysis of fecal coliform and/or nutrients. Samples are intended to be collected from surface water and stormwater monitoring sites with direct access to the flow stream or sample medium. It is intended that this document pertain only to surface water and stormwater sampling sites that allow access to the entire flow stream and/or when sampling is done just beneath the water's surface (i.e. up to 12 inches beneath the water's surface). If the flow stream is well-mixed and the chemistry is relatively uniform, the methods described in this SOP are sufficient to represent the water body. The procedures in this SOP may also be used to collect bacteria samples such as E. coli, Enterococci, etc. Standard Methods contains analytical procedures for laboratory analysis.

3.0 Definitions

Fecal coliform- A group of bacteria that inhabit the intestinal tract of warm-blooded animals and remain viable in freshwater for a variable period of time. The presence of fecal coliform bacteria in surface water indicates fecal contamination by a warm-blooded animal; harmful bacteria and viruses associated with fecal contamination may also be present.

4.0 Equipment and Materials

The following is a list of equipment for collecting surface water and stormwater grab samples.

- Sterile, plastic sample bottles (provided by lab)
- Extension sampler pole and/or bridge sampler and sampling ropes
- Cooler containing ice
- Field data sheet or field log book
- Chain of Custody form
- Site files detailing sampling locations, sample site information, site identification codes, and past site visits
- Orange safety vest for sites accessed from roadways
- Anti-bacterial hand sanitizer
- Latex gloves (recommended)

4.1 Sample Containers

The typical bacteria sample containers are 150mL or 250mL pre-autoclaved polypropylene bottles. Masking tape with black lines sealing the bottle lid indicates the bottle was autoclaved. Pre-autoclaved

bottles should not be used if the tape is missing or black lines are not present. The laboratory may provide one-time use sterile plastic bottles. These bottles will have a label sealing the lid and should not be used if the seal has been broken.

When chlorine is suspected to be present in the sample water, bottles with sodium thiosulfate (thiosulfate) added should be requested from the laboratory. Thiosulfate will not affect samples if chlorine is not present.

5.0 Procedures

The following procedures relate to the direct collection of surface water and stormwater samples with the sample container itself.

5.1 Field Preparation

1. Gather necessary field/sampling documentation and site-specific information.
2. Reference the project checklist and/or field file to determine appropriate number of sample bottles and assemble sampling/safety equipment.
3. Obtain sample bottles from the certified laboratory that will analyze the samples. Care should be used at all times to avoid contamination of the inside of the sample bottle or bottle cap. The sample needs to be placed in ice in a cooler as soon as possible after collection. *Note: Non-drinking water bacteria samples have a maximum holding time of 24 hours (APHA 2000).*
4. If the range of bacteria concentration can be estimated before sampling, let the lab microbiologist know so that a set of dilutions that bracket the expected concentration range can be prepared.

5.2 General Sampling Techniques

Bacteria sampling requires careful attention to sampling methods to avoid contamination of the bottle and water sample and ensure a representative sample is collected. The following guidance should be consistently considered:

1. Avoid contamination of the inside of the bottle cap and mouth. These should not be touched by hands or any other surface that may have bacteria exposure.
2. The bottle should not be rinsed or have water poured from another container that is not sterilized.
3. Avoid disturbing the sediment from the stream bed, particularly in slow moving waters.
4. Avoid sample collection from the surface layer (top inch of water column), near the streambank, and from eddies and side channels. In shallow depths, make notes on field form if a surface sample is unavoidable.
5. Avoid sample collection from stagnant waters (generally less than 0.1 ft/s) and eddies.
6. Collect samples from the active part of the stream where there is sufficient mixing to ensure the sample is representative.
7. If sample is collected from a boat, collect upstream of the boat's engine to avoid hydrocarbon contamination.

5.3 Sample Collection

Hand Dip Method: This method is typically used to collect samples within reach of the water surface (when standing in or near the stream or from a small boat).

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Move to a well mixed location, such as the deepest part of the active channel or another location where a representative sample may be collected. Do not contaminate the sample location by wading upstream of it. *Note: Use the Extension Pole Method if sampling from a lake or wide stream or river.*

3. Hold the base of the sample bottle with one hand and remove the bottle cap. Invert the bottle, reach upstream, and submerge the bottle into the water about 6 inches, and then tip the bottle mouth upstream and toward the water surface. Allow the bottle to fill to approximately the shoulder and take it out of the water. If the bottle is overfilled, immediately dump some water from the bottle. *Note: In shallow surface water, ensure that the sample bottle does not touch or disturb the stream bed, potentially contaminating the sample. Submerge the sample bottle to approximately the midpoint of the water column and tip upwards toward the direction of the flow. Samples should be collected far enough below the surface to avoid contamination from surface film and detritus. If a surface sample is unavoidable, note this on the field data sheet.*
4. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

Extension Pole Method: This method is typically used to reach a more representative or undisturbed sample location from the stream bank, or when sampling a lake or slow moving stream.

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Secure the sample bottle in the extension pole clamp.
3. Move to a location where a representative sample can be reached with the pole.
4. Remove the bottle cap avoiding contamination of the cap or inside of the bottle.
5. Position the bottle over the desired sampling location.
5. Invert the bottle and in one quick motion submerge the mouth of the bottle into the water column to a depth of approximately six inches. Slowly move the bottle upstream with the bottle mouth tipped toward the surface until the bottle fills to the bottle shoulder. For lake sampling, slowly move the tipped bottle away from the bottle entry point until it fills. If the bottle is overfilled, immediately dump some water from the bottle. *Note: In shallow surface water, ensure that the sample bottle does not touch or disturb the stream bed, potentially contaminating the sample. Submerge the sample bottle to approximately the midpoint of the water column and tip upwards toward the direction of the flow. Samples should be collected far enough below the surface to avoid contamination from surface film and detritus. If a surface sample is unavoidable, note this on the field data sheet.*
6. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

Hand Collection from Pipe Method: This method is typically used to collect samples within reach of the end of a stormwater pipe. A sample of stormwater discharge should be taken as a single uninterrupted event (i.e., grabbed at one time) from a single stormwater outfall.

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Move to the end of a stormwater pipe where there is moderate flow with turbulence, if possible, so the stormwater discharge will be well-mixed and representative. When sampling a stormwater system, samples should be sampled from the system discharge point first to ensure samples are not contaminated by upstream sampling.
3. Hold the base of the sample bottle with one hand and remove the bottle cap. Hold the bottle under the stormwater discharge with its opening positioned into the flow of water so that water enters directly into the bottle without flowing over the bottle or hands during sampling to prevent contaminating the sample.
4. Allow the bottle to fill to approximately the shoulder and take it out of the water. If the bottle is overfilled, immediately dump some water from the bottle. *Note: Ensure that the sample bottle does not touch the outfall pipe, potentially contaminating the sample.*
5. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

Bridge Sampling Method: This method is typically used to collect samples when standing on a bridge or boat.

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Secure the sample bottle in the bridge sampler and attach the sampling rope.
3. Move to a location where a representative sample can be reached with the sampler.
4. Remove the bottle cap avoiding contamination of the cap or inside of the bottle. Hold the cap with your free hand or set the cap upside down on a surface to avoid contamination of the inside of the cap (e.g. road, bridge, or clipboard).
5. Position the bottle and sampler over the desired sampling location. Lower the sampler to the water surface and allow the bottom of the sampler to touch the water surface to remove any debris from the bottom of the bottle and sampler. Raise the sampler off the water surface to allow debris to wash downstream. *Note: Ensure debris is not dislodged from the bridge while lowering the sampler. This step is intended to prevent sample contamination from any debris attached to the sampler.*
6. Without submerging the mouth of the bottle, lower the sampler into the water and allow the current to position the sampler so the bottle is on the upstream side. Rapidly lower the sampler so the mouth of the bottle to a depth of approximately 6 inches. The rapid motion is intended to minimize collection of the surface film. Allow the bottle to fill to approximately the shoulder and take it out of the water. If the bottle is overfilled, immediately dump some water from the bottle. *Note: In shallow surface water, ensure that the sampler does not touch or disturb the stream bed, potentially contaminating the sample. If a surface sample is unavoidable, note this on the field data sheet.*
7. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

5.4 Field Processing

1. Ensure the bottle label matches the desired sample site and includes the correct date and time.
2. Place the sample bottle in cooler.
3. Record the sample site ID, data, time of sample collection on the field data sheet and *Chain of Custody* form. Other notes on conditions of the sampling sites, adjacent land activities, or sample collection methods should be recorded on the field data sheet.

6.0 Records Management

Field sheets, chain of custody forms, and field/laboratory results will be stored in project files at Whatcom County Public Works- Natural Resources. After approximately six years, the documents will be boxed and archived. Laboratory results will be reviewed and verified. Data will be entered into an Excel spreadsheet for the project and saved on the Whatcom County network.

7.0 Quality Control and Quality Assurance

QA/QC procedures will be addressed thoroughly on a project-by-project basis in the Quality Assurance Project Plan (QAPP) or monitoring plan. General QA/QC procedures will include field notes, sample identification, representative sampling, field duplicates, comparability, and completeness. Personnel collecting water samples will have received training on the sampling protocol.

Data quality control consists of two elements: 1) Sample collection, handling, and storage will be conducted in a manner consistent with the methods outlined in the SOP and 2) field quality control samples will be collected with each sampling run. The field duplicate is a replicated sample collected immediately after the routine sample at the same location. One field duplicate (field quality control sample) will be collected in a side-by-side manner, per every "set" of samples to assess field sampling variability. A set equals 10 or fewer samples; thus 8 samples would be considered 1 set and 1 field duplicate would be collected, whereas 11 samples would be considered 2 sets

and 2 field duplicates would be collected. The site where the field duplicates are collected will be pre-determined before the sampling run using a random number table or generator. If there is not adequate flow to sample at the pre-determined site, the field duplicate will be sampled at the next site in the sampling run. Variability of field duplicates will be analyzed against pre-determined data quality objectives. Results exceeding objectives will be flagged and qualified or rejected.

Table 1. Summary of typical analytical methods for fecal coliform analysis.

Parameter	Description	Method	Sample Container	Preservation	Holding Time	Precision/Quantification Limits
Fecal coliform bacteria	Membrane filtration	SM9222D ¹	125mL or 250mL, sterile	10°C, dark	8 hours, maximum 24 (delivered to lab w/i 6 hrs, 2 hrs until sample processed)	20% RSD ² 1cfu/100mL

¹APHA et al. 2005. Standard Methods for the Examination of Water and Wastewater, 21st Edition.

² RSD- Relative standard deviation, divided by the mean

8.0 Safety

- Field staff will typically work in a team of two and carry a cell phone. In some circumstances, field staff will collect samples independently. In this case, it is essential that a cell phone is carried.
- High-visibility safety vests will be worn, particularly when working from or near a roadway.
- Field staff should use caution when approaching sampling sites. Sites may have slippery or unstable conditions. If field staff have concerns regarding accessing a site safely due to environmental conditions, construction, or other factors, the sample collection for that site should be aborted. The reason for not collecting a sample should be noted on the field sheet. If the condition is permanent, the site may need to be relocated.
- Gloves should be worn or hands washed after sampling surfaces waters.

9.0 References

City of Portland (Oregon). 2003. *Field Operations Standard Operating Procedures: Direct Grab Sample Collection with Sample Bottle*. Bureau of Environmental Services, Environmental Investigations Division.

Environmental Protection Agency (EPA). 1992. *NPDES Storm Water Sampling Guidance Document*. USEPA Office of Water. EPA 833-8-92-001.

Washington State Department of Ecology. 2007. *Standard Operating Procedures for the Collection of Fecal Coliform Bacteria Samples: Version 1.2*. Environmental Assessment Program.

Washington State Department of Ecology. 2011. *Standard Operating Procedures for the Collection of Fecal Coliform Bacteria Samples in Surface Water: Version 2.1*. Environmental Assessment Program.