

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedures for the Collection of Fecal Coliform Bacteria Samples in Surface water

Version 2.1

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EAP030

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Signatures on File.

This is a harmonized version combining SOP EAP030 and EAP012, which were both sample collection SOPs for fecal coliform bacteria.

*Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.*

*Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.*

*Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.*

SOP Revision History

Revision Date	Rev	Summary of changes	Sections	Reviser(s)
2/2/2007	1.0	Editorial; formatting	all	Bill Ward
2/9/2007		Internal Review	all	Dave Hallock
3/21/2007	1.1	Incorporate Comments, More Edits	all	Bill Ward
4/2/2007		QAO Review	all	Bill Kammin
4/5/2007	1.2	Incorporate Comments, More Edits	all	Bill Ward
4/18/2007		Final Review	all	Maggie Bell-McKinnon
4/22/2007	1.3	Minor Edits	all	Bill Ward
10/15/10	1.3	Recertified	All	Kammin
11/10/10	2.0	Harmonized with EAP SOP#012	all	Bill Ward; Nuri Mathieu
2/2/11	2.1	Incorporated comments from technical reviewers	all	Bill Ward; Nuri Mathieu

## Environmental Assessment Program

### Standard Operating Procedure for the Collection of Fecal Coliform Bacteria Samples

#### **1.0 Purpose and Scope**

- 1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) for the collection of freshwater samples for laboratory analysis of fecal coliform bacteria. The typical methods for fecal coliform analysis are: Standard Methods (SM) 9222D – a membrane filtration method and SM9221E 1 a most-probable number method using an EC medium. The procedures in this SOP may also be used to collect other bacteria samples such as E. coli, Enterococci, etc. Standard Methods contains alternative analytical procedures for these bacteria parameters.
- 1.2 This SOP includes the procedures for sample collection by hand, using a bacteria sampler, or with an extension pole. The SOP also covers sample collection from waters with high residual chlorine (treated effluent or receiving waters) or metals contamination.
- 1.3 Surface water can contain pathogenic (disease-causing) microorganisms. Testing water samples for the presence of all pathogenic microorganisms is expensive. Due to this high cost, Ecology tests water samples for fecal coliform bacteria, an organism used as an indicator of the potential presence of other pathogenic microorganisms.

#### **2.0 Applicability**

- 2.1 This SOP applies to the collection of bacteria samples in surface water.

#### **3.0 Definitions**

- 3.1 Ecology – Washington State Department of Ecology.
- 3.2 EAP – Environmental Assessment Program.
- 3.3 EIM – Environmental Information Management System. A searchable database developed and maintained by the Washington State Department of Ecology
- 3.4 Fecal coliform – A group of bacteria that inhabit the intestinal tract of warm-blooded animals and remain viable (alive and capable of infecting another organism) in freshwater for a variable period of time. The presence of fecal coliform bacteria in water indicates fecal contamination of the water by a warm-blooded animal; harmful bacteria and viruses associated with fecal contamination may also be present.

3.5 Field Logbook – A weather resistant logbook containing “Rite in the Rain” ® writing paper used to document any and all field activities, sample data, methods and observations for each and all collection sites.

3.6 MEL- Manchester Environmental Laboratory.

3.7 QA – Quality Assurance

#### **4.0 Personnel Qualifications/Responsibilities**

4.1 Field operations require training specified in EAP's Field Safety Manual (Ecology, 2006) such as First Aid, CPR, and Defensive Driving.

4.2 Boat operations require that staff meet specific training requirements as described in EAP’s Field Safety Manual, such as an EAP Boating Course and an approved Boating Safety Course.

#### **5.0 Equipment, Reagents, and Supplies**

##### 5.1 Supplies

5.1.1 Bacteria bridge sampler (Figure 1 – left)

5.1.2 Sampling ropes 1 @ 10 ft., 1 @ 35 ft. and 2 @ 55 ft.

5.1.3 Extension pole with bottle clamp (Figure 1 – right)

5.1.4 Cooler containing ice

5.1.5 250 or 500 mL autoclaved bacteria sample bottles<sup>1</sup> (Figure 1 – center)

5.1.6 Field Logbook or Field Data Report Form (see Attachment A for form)

5.1.7 Gloves (for sites where bacteria levels are known or are suspected to be high)

5.1.8 Antibacterial hand sanitizer

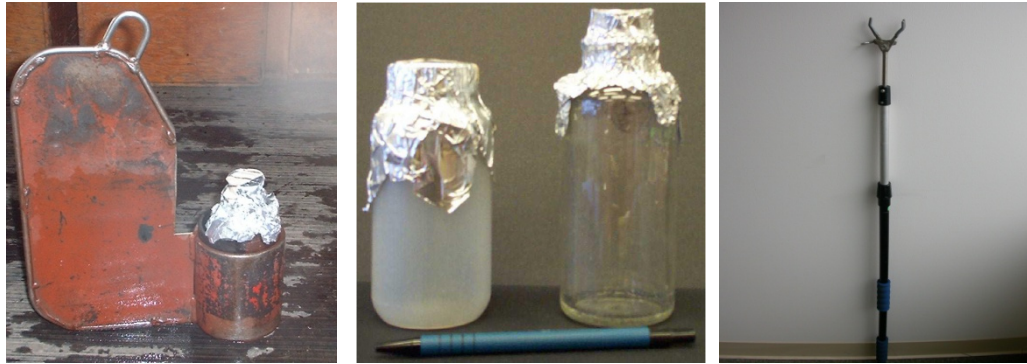
5.1.9 Pre-Sampling Notification (PSN) form

5.1.10 Lab Analysis Request (LAR) forms

5.1.11 Sample tags with pre-assigned sample numbers from the lab

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<sup>1</sup> 500 ml sample bottles may be necessary if both fecal coliform and other bacteria tests are conducted.



**Figure 1. Left: Bacteria bridge sampler with bottle. Center: 250 mL polypropylene and glass bottles. Right: Sampling extension pole.**

## 5.2 Sample Containers

5.2.1 The typical bacteria sample containers are 250-mL or 500mL pre-autoclaved polypropylene bottles with aluminum foil wrapped caps used to preserve sterility near the bottle opening (Figure 1-center). *Note: Masking tape with black lines on the foil mean the bottle was autoclaved. These bottles should not be used after 6 months.*

5.2.2 When chlorine is suspected to be present in the sample water, bottles with sodium thiosulfate (thiosulfate) added should be requested from the laboratory. The bottles will be marked with yellow stickers on top to indicate the thiosulfate addition. Thiosulfate will not affect samples if chlorine is not present.

5.2.3 If heavy metal contamination ( $>1.0$  mg/L of Copper or Zinc) is suspected, bottles must have EDTA added. This is a special request and must be set up through the laboratory.

## 6.0 **Summary of Procedure**

### 6.1 Field Preparation

6.1.1 Before the start of a new project, the lab must review and approve the project specific Quality Assurance Project Plan (QAPP). Before each sampling run, a Pre-Sampling Notification form must be submitted to the lab at least two weeks prior to any sampling. This ensures the lab has adequate time to prepare the medium for bacterial analysis. *Note: Avoid bacteria sample collection between Thursday and Sunday. Sample collection on these days must be pre-approved with the lab, but is possible under some special circumstances.*

6.1.2 If the range of bacteria concentrations 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.

6.1.3 Under certain conditions the most probable number (MPN) method is recommended: 1) if the sample is collected from extremely turbid water ( $<25$  mL can be filtered); or 2) if the sample is collected in or near a WA Department of Health (DOH) shellfish growing area. The lab needs additional time to prepare for the MPN method, so sample notification should be provided at least one month in advance.

6.1.4 Prior to collecting samples prepare sample tags prepared containing the project name, sample number, site, date, and space for time.

## 6.2 General Sampling Techniques

6.2.1 Care should be used at all times to avoid contamination of the inside of the sample bottle, or the foil-covered silicon stopper or bottle cap. Also, 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).*

6.2.2 Do not rinse the bottle and do not pour water into a bacteria bottle from another non-sterilized container.

6.2.3 Be careful not to disturb sediment from the stream bed, particularly in slower moving waters. For slow moving streams with easily disturbed sediment, collect the sample from the stream bank using a sampling extension pole (Figure 1) or from a bridge with a bacteria bridge sampler

6.2.4 Always: 1) Collect samples from the active part of the stream (thalweg) to ensure the sample is representative of the waterbody; and 2) Face the opening of the bottle upstream (or into the tidal flow in marine water).

6.2.5 Avoid sample collection: 1) from the surface layer (top inch of water); 2) near the streambed; and 3) from back eddies and side channels. *Note: In extremely shallow depths, collect the sample from the surface if unavoidable and record in field notes.*

6.2.6 When filling the sample bottle, be careful to pull the bottle out of the water as it reaches the point where it is filled to at or near the shoulder of the bottle. If the bottle becomes filled above this level, then immediately pour out excess sample downstream of sampler. *Note: This step allows air space for proper mixing at the lab.*

6.2.7 If on a boat, then collect samples upstream of the engine cooling system to avoid any potential gas and oil contamination.

6.2.8 If the samples need to be collected in slow moving waters with stratified velocity, then collect a depth-integrated bacteria sample. To collect a depth-integrated sample, first submerge the bottle (mouth facing down) to roughly 25 percent of the water's depth. Next, invert the bottle slightly until it begins to fill and then slowly move the bottle up through the water column as it fills. Quickly remove the bottle from beneath the water when the bottle reaches roughly 75 percent of the water's depth and the water level inside the bottle is at or near the shoulder. *Note: Depth integrated bacteria sampling is performed in TMDL or other special studies.*

- 6.2.9 Avoid sample collection in stagnant waters. If unsure whether or not water is stagnant (generally less than 0.1 ft/s), then use a flow meter to measure velocity. *Note: TMDL or other special studies may require sample collection in stagnant water under certain conditions (e.g. sampling behind a pump station or tide gate).*
- 6.2.10 If sample bottles contain an additive (thiosulfate or EDTA), then either: 1) collect sample with a sterile bottle without additive and pour mixed sample into bottle with additive; or 2) follow instructions for Hand Dip Method for Waters with High Chlorine Content or Metals Contamination (6.3.3).
- 6.2.11 If sample bottles with additive are accidentally overfilled pour the excess out. Manchester Environmental Laboratory (MEL) adds the additive to the bottles in excess. MEL qualifies the sample results for overfilled bottles as “bottle overflow.”
- 6.2.12 If sampling wastewater or industrial effluent, then locate an appropriate sampling location representative of water being discharged to the receiving water body. In particular, the location should be below any chlorination or ultra-violet (UV) application and as close to the discharge outfall as possible. Wear gloves and use a sampling extension pole to collect samples without contacting the effluent with your hands

### 6.3 Sample Collection

- 6.3.1 Bridge Sampler Method.** This method is typically used to collect samples when standing on a bridge or boat.
- 6.3.1.1 Secure the bacteria sample bottle into the bacteria sampler and attach the sampling rope.
- 6.3.1.2 Move to a well mixed, representative location such as the deepest part of the active channel.
- 6.3.1.3 Remove the aluminum foil covered stopper or lid, taking care not to touch the inside or bottom of the lid, and either 1) hold onto it with a freehand or 2) set it upside down where contamination to the inside of the lid can be avoided (such as on the road surface or on top of other field equipment). *Note: In windy conditions, set the lid down somewhere shielded from the wind so that it doesn't blow over (such as on the leeward side of sampling equipment.)*
- 6.3.1.4 Carefully lower the sampler to the water surface, taking care to not dislodge bridge debris onto it. Allow the bottom of the sampler to touch the water surface, then raise the sampler off the water for a few moments to allow any debris from the bottom of the sampler to drop off and float away. *Note: This step is intended to prevent sample contamination from anything attached to bottom of the sampler.*

- 6.3.1.5 Without submerging the bottle mouth, lower the sampler about 15 cm (6 inches) into the water. Allow the current to re-orient the sampler so the sample bottle is on the upstream side of the sampler. Then rapidly lower the sampler about 0.5 meters to completely submerge it. *Note: This minimizes the sampling of surface film.*
- 6.3.1.6 Retrieve the filled bottle taking care to not dislodge bridge debris onto it or the sampler.
- 6.3.1.7 If the bottle is filled above the shoulder, then immediately pour out enough excess sample to ensure that the sample volume is at or near the shoulder.
- 6.3.1.8 Carefully replace the aluminum foil covered stopper or lid. Make sure the foil is open wide enough to avoid touching it to the top or inside of the bottle. Also, do not touch the lower part of the stopper or inside of the cap with your hand.
- 6.3.2 Hand Dip Method.** This method is typically used to collect samples within reach of the water surface (when standing in or near the waterbody or from small boat).
- 6.3.2.1 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. If collecting from an eddy, then do not wade anywhere in the eddy prior to sample collection. *Note: The Extension Pole Method (see 6.3.4 below) should always be used to collect lake samples.*
- 6.3.2.2 Remove the lid and set it upside down following section 6.3.1.3.
- 6.3.2.3 Grab the base of the sample bottle with one hand and remove the bottle cap. Then invert the bottle, reach upstream, and plunge the bottle into the water about 15 cm (6 inches), and then tip the bottle mouth up toward the water surface. Allow the bottle to fill and then take it out of the water. *Note: If sampling from a boat, then plunge the bottle into the water upstream of the engine, and move it away from the boat while and tipping the bottle mouth up toward the water surface.*
- 6.3.2.4 If the bottle is filled above the shoulder, then immediately pour out enough excess sample to ensure that the sample volume is at or near the shoulder.
- 6.3.2.5 Replace the lid as described in section 6.3.1.8.
- 6.3.3 Hand Dip Method for Waters with High Chlorine Content or Metals Contamination.** This method is used when the sample container contains thiosulfate (for chlorine) or EDTA (for metals) preservative. In lieu of this method, the sample may be collected in a sterile container without preservative, using any other method described in this SOP, and then immediately transferred to the container with preservative.
- 6.3.3.1 Remove the stopper/lid from the bottle just before sampling and set the aluminum foil down following section 6.3.1.3

- 6.3.3.2 Place the lid over the mouth leaving a small opening on one side.
- 6.3.3.3 Place one hand around the bottle with a finger holding the lid in place.
- 6.3.3.4 Quickly plunge bottle (mouth facing up) through the surface layer with the top of the bottle tilted forward and the opening facing upstream.
- 6.3.3.5 Keep the bottle submerged long enough for the bottle to fill to at or near the shoulder of the bottle. Try to avoid overfilling the bottle.
- 6.3.3.6 Quickly remove bottle from water to avoid the surface layer
- 6.3.3.7 If the bottle is filled above the shoulder, then immediately pour out enough excess sample to ensure that the sample volume is at or near the shoulder.
- 6.3.2.6 Replace the lid as described in section 6.3.1.8.
- 6.3.4 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.
  - 6.3.4.1 Secure the bacteria sample bottle in the extension pole clamp.
  - 6.3.4.2 Move to a location where a representative sample may be reached with the pole.
  - 6.3.4.3 Remove the lid and set it upside down following section 6.3.1.3
  - 6.3.4.4 Position the bottle over the desired sample location.
  - 6.3.4.5 Invert the bottle and in one quick motion plunge the mouth of the bottle into the water about 15 cm (6 inches). Then slowly move the bottle upstream with the bottle mouth tipped toward the water surface until the bottle fills. If sampling a lake, then slowly move the tipped bottle away from the bottle entry point until it completely fills.
  - 6.3.4.6 Take the bottle out of the water. If the bottle is filled above the shoulder, then immediately pour out enough excess sample to ensure that the sample volume is at or near the shoulder.
- 6.3.2.7 Replace the lid as described in section 6.3.1.8.
- 6.3.5 Bathing Beach Method.** This method is used when collecting samples from bathing beaches. Section 6.3.5 was taken from the Beach Environmental Assessment, Communication and Health (BEACH) program guidance. More beach sampling information is available from the: [Quality Assurance Project Plan: BEACH Program](#) (Schneider, 2004).

- 6.3.5.1 Wade into roughly 2.5 feet of water.
- 6.3.5.2 Fill a bottle at each of the three sampling sites by wading into knee deep water, unscrewing the cap and inserting the bottle and cap into the water at a 45 degree angle (with the bottle opening facing down). Turn the bottle upright a few inches below the surface and allow it to fill. Remove the cap and bottle from the water and pour off enough water to leave an air space. Cap the bottle. Use a sampling extension pole to avoid collecting disturbed sediment.
- 6.3.5.3 Deliver to laboratory within six hours of sample collection.

## 6.4 Field Processing

- 6.4.1 Label the collected sample bottle with the appropriate tag and place it in ice in a cooler to preserve the sample during shipment to the laboratory.
- 6.4.2 Record the date, time, and sampling location on a Field Logbook or Field Data Report Form.

## 7.0 **Records Management**

- 7.1 All hardcopy documentation of the data, such as completed Field Logbook and Field Data Report Forms are kept and maintained by the project lead. These documents are organized in binders or in expanding files. After about six years, hardcopies are boxed and moved to EAP archives.
- 7.2 Data collected for Ecology's Ambient River and Stream Monitoring Program will be entered into an Access-based database, reviewed and verified following the Quality Control and Quality Assurance procedures (see 8.1 below), uploaded into EIM, and posted on this web page [http://www.ecy.wa.gov/programs/eap/fw\\_riv/rv\\_main.html](http://www.ecy.wa.gov/programs/eap/fw_riv/rv_main.html).
- 7.3 Data collected for special project or Total Maximum Daily Load (TMDL) studies will be reviewed, verified, and stored based on the QAPP for the project.

## 8.0 **Quality Control and Quality Assurance**

### 8.1 Freshwater Ambient Monitoring Program

- 8.1.1 The data QA program for field sampling consists of two parts: (1) adherence to the SOP procedures for sample/data collection and periodic evaluation of sampling personnel and (2) the collection of a field quality control (QC) sample during each sampling run. The Freshwater Ambient Monitoring QA program is described in detail in [www.ecy.wa.gov/biblio/0303200.html](http://www.ecy.wa.gov/biblio/0303200.html) (Hallock and Ehinger 2003).

8.1.2 Each run one field QC sample is collected as a duplicate sequential field sample and one 500mL sample is collected for a laboratory split sample. The duplicate sample consists of the collection of an additional sample approximately 15-20 minutes after the initial collection at a station. This sample represents the total variability due to short-term, in-stream dynamics, sample collection and processing, and laboratory analysis.

8.1.3 A two-tiered system will be used to evaluate data quality of individual results based on field QC. The first tier consists of an evaluation of the variability in field duplicates and the reasonableness of the result. Results exceeding pre-set limits are flagged. The second tier QC evaluation is a manual review of the data flagged in the first tier. Data are then coded from 1 through 9 (1 = data meets all QA requirements, 9 = data are unusable). Criteria for assigning codes are discussed in more detail in Hallock and Ehinger (2003). Do not routinely use or distribute data with quality codes greater than 4. *Note: results from highly turbid samples are estimated.*

## 8.2 Total Maximum Daily Load (TMDL) Studies

8.2.1 The QA process for TMDL field samples consists of two parts: (1) adherence to the SOP procedures for sample/data collection and periodic evaluation of sampling personnel and (2) the collection of a field QC sample for twenty percent of the samples collected for a given study. The TMDL QA process is described in greater detail in Lombard and Kirchimer (2004).

8.2.2 The field QC sample is collected as a duplicate sample in either a side by side manner or immediately following the initial sample. This sample represents the total variability due to sample collection and laboratory analysis.

8.2.3 Recommendations for evaluating precision from bacteria duplicate results can be found in Mathieu (2006).

8.2.4 QA/QC procedures are addressed on a project-by-project basis in the QAPP for the project.

## 9.0 Safety

9.1 Safety is the primary concern when collecting samples. Since many sample sites are located near roads and bridges, road and pass conditions should always be checked before departure (especially in winter). If roadside hazards, weather, accidents, construction, etc. make sample collection dangerous, then skip that station or sampling run if necessary. Note the reason on the Field Data Report Form and notify your supervisor of the hazard when you return to the office. If the hazard is a permanent condition, relocation of the station may be necessary. Review Ecology's Safety Program Manual periodically to assist with these safety determinations (Ecology, 2010).

- 9.2 Gloves should be worn to avoid exposure. If gloves are not worn, hands should be cleaned using anti-bacterial soap or hand sanitizer after completing work at each sampling station or, at a minimum, after completing work at sampling stations with known high bacteria counts and before ingesting food or drink.
- 9.3 After sampling assume your hands and anything they touch are contaminated with bacteria and use care accordingly.

## 10.0 References

- 10.1 APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater-. No: 4500-O C. Winkler Method, Azide Modification, American Public Health Association, 21<sup>st</sup> Edition. Washington D.C.
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<http://aww.ecology/services/es/Safety/ChemicalHygiene.pdf>
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<http://aww.ecology/programs/eap/Safety/Safety1.html>
- 10.4 Hallock, D. and W. Ehinger, 2003. Quality Assurance Monitoring Plan: Stream Ambient Water Quality Monitoring. Washington State Department of Ecology, Olympia, WA. 27pp. Publication No. 03-03-200.  
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<http://www.ecy.wa.gov/biblio/0603044.html>
- 10.7 Schneider, L., 2004. Quality Assurance Project Plan: BEACH Program. Washington State Department of Ecology, Olympia, WA. 21pp. Publication No. 04-03-205.  
<http://www.ecy.wa.gov/biblio/0403205.html>

**ATTACHMENT A**

**FIELD DATA REPORT FORM**



