

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedure for Sampling Bacteria in Water

Version 1.0

Author – Nuri Mathieu

Date -

Reviewer – Karol Erickson, Water Quality Studies Supervisor

Date -

QA Approval – Bill Kammin, Ecology QA Officer

Date -

Program Approval – Will Kendra, Watershed Ecology Section Manager

Date -

EAP012

PROVISIONALLY APPROVED: June 26, 2006

Signatures on File

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.

Environmental Assessment Program

Standard Operating Procedure for Sampling Bacteria

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Water Quality Studies Unit Standard Operating Procedure (SOP) for Sampling Bacteria. The most common type of bacteria sampled for is fecal coliform; however, this SOP also covers E. coli and other types of bacteria.

2.0 Applicability

2.1 This SOP should be followed for all sampling for bacteria in water. It includes procedures for collecting grab samples from a wadable stream and from boats, sampling from a bridge, collecting samples from wastewater treatment plant effluent, and collecting samples from swimming beaches.

3.0 Definitions

3.1 Lotic: Flowing water systems such as rivers and streams.

3.2 Thalweg: The line defining the lowest points along the length of a river bed.

4.0 Personnel Qualifications/Responsibilities

4.1 No special qualifications required.

5.0 Equipment, Reagents, and Supplies

5.1 Supplies

5.1.1 250 or 500 mL autoclaved glass or polypropylene bottle.

5.1.2 Latex gloves (for sites where bacteria level is known or suspected to be high).

5.1.3 Anti-bacterial hand sanitizer or soap.

5.1.4 Cooler/s.

5.1.5 Ice (Regular, blue, or dry –depending on shipping method).

5.1.6 Tap water. Sample tags with sample numbers assigned by MEL.

5.1.7 Lab Analysis Request (LAR) forms.

5.1.8 Sampling extension pole.

5.1.9 Specialized bridge sampler (if applicable).

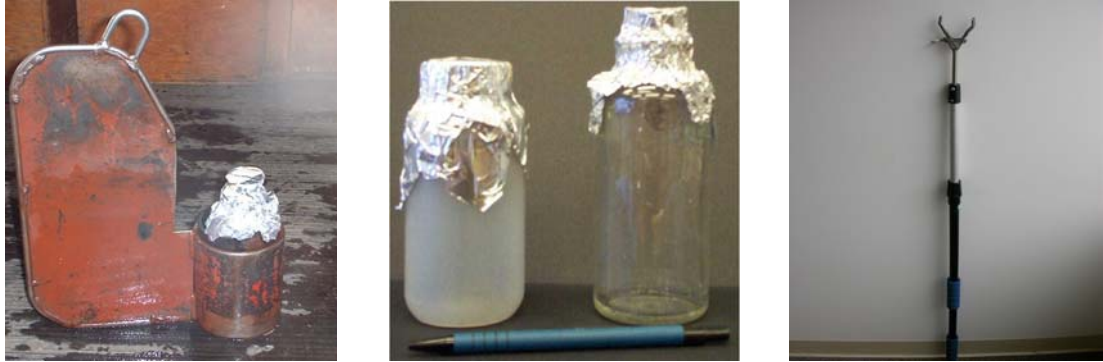


Figure 1. Left: Specialized bridge sampler with bottle. Center: 250 mL polypropylene and glass bottles. Right: Sampling extension pole.

5.2 Sample Containers

5.2.1 The normal container for bacteria sampling is a 250-mL pre-autoclaved glass bottle (with cork stopper) or polypropylene bottle and cap. The bottle normally comes from the lab with aluminum foil wrapped over the cover to preserve sterility (Figure 1).

5.2.2 When chlorine is suspected to be present, bottles with thiosulfate added should be requested from the laboratory. The bottles will be marked with yellow stickers on top to indicate the sodium thiosulfate addition. Thiosulfate will not affect samples if chlorine is not present. The stopper should have masking tape with black lines, which means the bottle has been autoclaved.

5.2.3 If high metal contamination is suspected, bottles must have EDTA added. This is a special request and must be set up through the laboratory.

5.2.4 Holding times for bottles:

5.2.4.1 3 months without thiosulfate/EDTA

5.2.4.2 1 month with thiosulfate/EDTA

5.2.5 500 mL autoclaved bottles should be used when collecting a sample for multiple bacteria parameters. For example, if analysis for both Fecal Coliform and E. coli is required, then one sample may be collected in a 500 mL bottle, rather than two separate 250 mL samples or one 250 mL sample (250 mL does not provide enough sample to analyze for multiple parameters).

6.0 Summary of Procedure

6.1 Sample Collection

6.1.1 General Procedures and Techniques

- 6.1.1.1 The laboratory needs to prepare medium for bacterial analysis. It is especially important that sampling is prearranged with the lab. Two weeks is the preferred amount of notice.
- 6.1.1.2 Sampling for bacteria on Thursday through Sunday must be pre-approved with the laboratory.
- 6.1.1.3 If the range of bacteria concentrations can be estimated before sampling (from past samples or otherwise), let the lab microbiologist know beforehand or on the sample tags so the proper set of dilutions can bracket the range.
- 6.1.1.4 Since there are two possible methods (Membrane Filter, MF, and Most Probable Number, MPN), record which method is to be used in the proper fields on the Pre-Sampling Notification (PSN) form, as well as on the Laboratory Analysis Requested (LAR) form. Both forms can be located in the MEL Lab User's Manual (MEL, 2005) .
- 6.1.1.5 If the water is extremely turbid (<25 mL can be filtered) the MPN method is necessary. Call the lab as soon as possible so they can prepare for this method.
- 6.1.1.6 General techniques and procedures apply to sampling special sources such as stormwater and combined sewer overflows. The Quality Assurance Project Plan (QAPP) may contain further guidance on sample planning and techniques for special circumstances.
- 6.1.1.7 Prior to collecting sample, have sample tag prepared containing the project name, sample number, site, date, and space for time. Also have field lab book or page prepared with similar information.
- 6.1.1.8 Do not rinse the bottle and do not pour water into a bacteria bottle from another non-sterilized container.
- 6.1.1.9 Face the opening of the bottle upstream and collect sample from thalweg or predominant flow. Avoid back eddies and side channels in lotic systems.
- 6.1.1.10 Collect sample at approximately 40 to 60 percent of the water's depth. Avoid collecting sample near the stream bed or surface layer. In shallow depths, collect sample from surface if unavoidable and record in field notes. Do not to let the bottle touch the stream bed.

- 6.1.1.11 When possible do not collect samples from stagnant waters. If unsure whether or not water is stagnant, use flow meter to measure velocity. Try not to collect samples from water where velocity is less than 0.1 feet per second. In some situations (e.g. sampling behind a pump station or tide gate) sampling stagnant water may be unavoidable.
- 6.1.1.12 Be careful not to disturb sediment from the stream bed, particularly in slower moving waters. For slow moving streams with easily disturbed sediment, collect sample from stream bank using sampling extension pole (Figure 1).
- 6.1.1.13 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 is filled above this level, immediately pour out (downstream of sampler) enough of sample so that the water level is at or near the shoulder of the bottle. This will allow enough air space above the sample for proper mixing before analysis at the lab.
- 6.1.1.14 For samples with preservative, if overfilling does occur the sample is not lost. The thiosulfate and EDTA that MEL adds to the bottles is in excess. Pour excess sample out of overfilled bottles with preservative, otherwise the sample result will be qualified as “bottle overflow.”
- 6.1.1.15 If sampling from a boat, avoid gas and oil contamination. Hold the bottle upstream while the boat moves forward.
- 6.1.1.16 Place labeled sample bottle in cooler. It is important to cool to 4°C immediately and store in dark cooler, as bacteria samples are sensitive to light.
- 6.1.2 Grab samples (Without Preservative)
 - 6.1.2.1 Remove stopper/lid from bottle just before sampling, leaving the aluminum foil over stopper/lid. Be careful not to contaminate the cork (glass bottle), cap (plastic bottle) or the inside of the bottle with your fingers, dirt particles, or dripping water from bridges, or other sources of contamination.
 - 6.1.2.2 Remember, DO NOT RINSE the bottle.
 - 6.1.2.3 Hold the bottle near its base, plunge it (mouth down) below the surface, avoiding the top micro-layer where bacteria tend to concentrate.
 - 6.1.2.4 While under water, turn the bottle into the current and away from you, the shore, and the side of the sampling platform or boat.
 - 6.1.2.5 Fill the bottle to the appropriate level (see techniques above).
 - 6.1.2.6 Securely replace the aluminum covered stopper/lid.
 - 6.1.2.7 Rinse any large amount of dirt or debris from the outside of the container

- 6.1.3 Grab samples (with preservative).
 - 6.1.3.1 Remove stopper/lid from bottle just before sampling and set the aluminum foil somewhere free of dirt or other sources of contamination.
 - 6.1.3.2 Place the lid over the mouth leaving a small opening on one side.
 - 6.1.3.3 Place hand around bottle with one finger holding lid in place.
 - 6.1.3.4 Quickly plunge bottle (mouth facing up) through the top micro-layer with the top of the bottle tilted forward and the opening facing upstream.
 - 6.1.3.5 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.
 - 6.1.3.6 Quickly remove bottle from water to avoid surface layer.
 - 6.1.3.7 Immediately pour out excess sample if bottle was overfilled.
 - 6.1.3.8 Securely replace the aluminum covered stopper/lid.
 - 6.1.3.9 Rinse any large amount of dirt or debris from the outside of the container.
- 6.1.4 Samples Collected using Specialized Bridge Sampler (Figure 1)
 - 6.1.4.1 Remove stopper/lid just before lowering the sampler-with-bottle down on the rope. Hold the stopper/lid via the aluminum foil, or set it somewhere free of dirt or other sources of contamination.
 - 6.1.4.2 Lower the sampler in such a manner so as not to contaminate the open bottle with dirt or dripping water.
 - 6.1.4.3 When approaching the water surface, drop the sampler quickly through the surface to avoid the micro-layer.
 - 6.1.4.4 Keep the bottle submerged long enough for the bottle to fill (or 1-2 inches below the top).
 - 6.1.4.5 Pull up the sampler and bottle, careful not to contaminate the sample with dirt or water from either the rope or bridge, or other sources of contamination.
 - 6.1.4.6 Pour out sample to allow for the air space needed for proper mixing at the lab (unless bottle contains preservative).
 - 6.1.4.7 Securely replace the aluminum covered stopper/lid.
 - 6.1.4.8 Rinse any large amount of dirt or debris from the outside of the container.

- 6.1.5 Samples collected from wastewater/point source effluent.
- 6.1.5.1 Prior to sampling request MPN method for chlorinated effluent.
- 6.1.5.2 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.
- 6.1.5.3 Use a sampling extension pole (Figure 1) to collect samples without contacting the effluent with your hands.
- 6.1.6 Samples collected from marine water bathing beach (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).
- 6.1.6.1 Wade into roughly 2.5 feet of water.
- 6.1.6.2 Fill a water bottle at each of 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. If possible use a sampling extension pole (Figure 1) to avoid collecting disturbed sediment.
- 6.2 Sample Labeling and Storage
- 6.2.1 After collecting sample, immediately loop the string attached to the sample tag over stopper/lid until secure (at least three loops for 250 mL and at least two loops for 500 mL). Make sure to attach sample tag beneath, not on top of, the aluminum foil cover, as the covers can be easily separated from the sample during transport and handling.
- 6.2.2 Record the time each station was sampled on the sample tag and in the field notes.
- 6.2.3 Samples collected to determine compliance with National Pollutant Discharge Elimination System (NPDES) permits must be analyzed within 6 to 8 hours. Samples collected for the BEACH Program should be delivered to the laboratory within six hours of collection. Samples of ambient and contaminated water should be analyzed within 6 to 8 hours (APHA, 1998); however, due to the logistics of sampling over the course of a day, MEL allows a 24 hour holding time (MEL, 2005). A holding time study may be in order for some studies.
- 6.3 Sample Transport
- 6.3.1 Samples transported from the EAP Operations Center (OC) by MEL courier

6.3.1.1 Samples transported via MEL courier may be collected in glass bottles, however be careful when handling and packing. Pack samples in regular cubed or crushed ice. Deliver samples to walk-in cooler at EAP OC and leave Lab Analysis Requested (LAR) forms in the “Out” box near the walk-in cooler. Make sure the LAR form contains the project name, station names, sample numbers, date, times, and parameters at a minimum.

6.3.2 Samples shipped via air or ground freight service

6.3.2.1 Samples must be collected in polypropylene containers, not glass. Pack samples using blue or dry ice (check with airline for restrictions on dry ice). Cool to 4°C and store in dark cooler. In warmer weather (80°F and above) use ten to twelve blue ice packs per cooler. In cooler weather (below 80°F) use six to eight blue ice packs, to avoid freezing samples. Tape Lab Analysis Requested (LAR) form to the inside of the cooler and tape coolers shut after inspection.

7.0 Records Management

8.0 Quality Control and Quality Assurance

8.1 QA/QC procedures will be addressed thoroughly on a project-by-project basis in the QAPP for the project.

9.0 Safety

9.1 Preferably, 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.

9.2 After sampling assume your hands and anything they touch are contaminated with bacteria and use care accordingly.

9.3 For further field health and safety measures refer to the: [Environmental Assessment Program \(EAP\) Safety Manual](#)

10.0 References

10.1 APHA, 1998. Standard Methods for the Examination of Waste and Wastewater. 20th Edition.

10.2 MEL, 2005. Manchester Environmental Laboratory Lab Users Manual Eighth Edition. Environmental Assessment Program. Washington State Department of Ecology. Manchester, WA.

10.3 Schneider, Lynn, 2004. Quality Assurance Project Plan: BEACH Program. Environmental Assessment Program of the Washington State Department of Ecology. Olympia, Washington.