

Standard Operating Procedure #004





Bacteria Sample Collection

Version 1.0
October 2018

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Natural Resources Department
Lummi Indian Business Council

Prepared for:
EPA Region 10

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This project has been funded wholly or in part by the United States Environmental Protection Agency under Assistance Agreement BG-00J89601-1 to the Lummi Nation. The contents of this document do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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REVISION RECORD

Approval	Date	Responsible Person	Description of Change	Location of Change
1	October 2018	Kara Kuhlman	Initial Approval and Release of Version 1.0	N/A
2				
3				

SIGNATURE PAGE

Document: Bacteria Sample Collection SOP #004

Version 1.0

The following technical staff have read this manual. A copy of this page will be distributed to the employee training record file.

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1. INTRODUCTION

This document, the Standard Operating Procedures (SOP) for bacteria (*i.e.*, fecal coliform, *Escherichia coli*, and *Enterococcus*) sample collection, should be used any time a bacteria sample is collected. This document describes the standard operating procedures and best practices for bacteria sample collection, including selection of a representative location, equipment needed, sterile technique, and appropriate sample collection procedures. This document is to be used in conjunction with the relevant project Quality Assurance Project Plan (QAPP) and instructions from the laboratory at which the sample will be analyzed.

1.1 Method Summary

Bacteria samples are collected in containers prepared and provided by an independent, contracted laboratory using instructions provided by the laboratory and summarized in this SOP. Bacteria samples are collected using sterile techniques by field staff, stored on ice, and transported to the appropriate laboratory for analysis. Membrane filtration, multiple tube dilution, presence/absence analyses, and/or colony enumeration are conducted by a certified laboratory. Quality assurance/quality control (QA/QC) procedures include chain of custody forms, field blanks, temperature controls, maximum holding times, and laboratory QA/QC procedures.

1.2 Health and Safety Warnings

No water quality measurement is worth risking injury or death. Field personnel must be aware of the environment, use common sense and training, and not exceed their abilities or limits. Field work is never conducted alone. All Lummi Natural Resources (LNR) Water Resources Division (LWRD) Health and Safety Plan (LWRD 2015a) requirements and guidelines are followed at all times while conducting fieldwork.

During bacteria sample collection, field personnel are exposed to water that is potentially contaminated with bacteria and other pathogens. Although coliforms are not usually pathogenic themselves, their presence is an indicator of potential pathogenic bacterial contamination. Field personnel must use good hygiene and good sense in undertaking sampling activities. Rubber or latex gloves are available for use if desired. Hands are washed thoroughly with soap and water or appropriate sanitary wipes as soon as possible after sampling at sites that may be contaminated with biological pathogens, and again prior to eating or drinking. Sampling equipment are rinsed and dried after use.

1.3 Quality Assurance/Quality Control (QA/QC)

Quality assurance/quality control activities include chain of custody forms, field blanks, temperature controls, holding times, sterile techniques, and laboratory QA/QC procedures.

1.4 Range, Accuracy, Readability

The range, accuracy, and readability of laboratory measurements are not presented in this SOP as they vary with the matrix and interfering compounds. Lower and upper detection limits are reported by the laboratory, and summarized in Table 4.1.

2. PREPARATION, EQUIPMENT, AND SITE CONDITIONS

This section describes the equipment needed to collect a bacteria sample, how to determine if a site is representative for collection of a sample, and how to minimize site disturbance.

2.1 Equipment

The following equipment are needed for collection of bacteria samples:

- Unopened and sterilized bacteria sample bottles (provided by the laboratory)
- Blue sampling wand
- Ice chest with ice
- Pen or permanent felt-tip marker (*e.g.*, Sharpie)
- Bottle of sterile water for first bacteria sample collection of the quarter (provided by the laboratory)
- Chain of custody form
- Additional equipment for delivery of Department of Health samples:
 - Polystyrene cooler
 - Shipping box
 - Reusable ice packs
 - Pre-paid shipping form
 - Plastic zip lock bag

2.2 Sample Bottles

Sterile sample bottles are provided by the laboratory at which the bacteria samples will be analyzed. As of the writing of this SOP, the LNR independently contracted laboratory for most surface water and groundwater bacteria analysis is Edge Analytical, Inc. (Edge) located in Bellingham, WA. Sample bottles provided by Edge are sterilized in an autoclave, and have autoclave tape with black diagonal stripes along the side of the lid (Figure 2.1).

All marine bacteria samples collected in partnership with the Department of Health (DOH) for the National Shellfish Sanitation Program (NSSP) are analyzed by the DOH Public Health Laboratory, located in Shoreline, WA. DOH-provided bacteria sample bottles have manufacturer-sealed lids (Figure 2.2).



Figure 2.1 Example of Bacteria Sample Bottle Provided by Edge Analytical with Autoclave Tape Across Side of Lid



Figure 2.2 Example of Bacteria Sample Bottle Provided by Department of Health with Manufacturer-Sealed Lid

2.2.1 Inspection of Bottles

Laboratory-supplied bottles are inspected upon receipt from the laboratory and prominently marked with an “X” on the bottle label and lid if:

- The bottle cap is off or loose
- The cap or bottle are damaged
- Autoclave tape is not present (Edge-provided bottle only)
- Autoclave tape does not show black diagonal stripes (Edge-provided bottle only)

Undamaged autoclave sample bottles can be returned to Edge for re-sterilization. Damaged bottles, bottles missing lids, and DOH-provided bottles with broken seals are disposed of in the LNR laboratory garbage or recycled if they do not contain hazardous materials. Laboratory-supplied bottles are not rinsed prior to sample collection unless specifically indicated by the laboratory. However, if the outside of laboratory-supplied bottles are dirty, the outside can be rinsed off in the sink at the LNR wet lab and dried with clean paper towels prior to sample collection. All laboratory bottles are securely stored in an upright position prior to and after use.

Bottles that are used in sampling unsuccessful water samples are marked with an “X” on the label and lid, and are not used for a second attempt at collection of a successful sample.

2.2.2 Sample Bottle Labeling

Laboratory-supplied bacteria sample bottles are labeled using a permanent marker or waterproof pen prior to collecting a water sample. Included on the sample label are sample site identifier, date, time (using 24-hour format), laboratory analysis requested, and agency collecting the sample. See Figure 2.3 for an example bottle label.

LOG #		
CLIENT	LIBC	DATE 2/13/12
		TIME 10:05
LOCATION	SW055	PRESERVATIVE ICE
ANALYSIS	Bacteria	

1500 North State Street, Bellingham, WA 98225

Figure 2.3 Example Bacteria Sample Bottle Label for Analysis at Edge Analytical

For samples to be delivered to the DOH Public Health Laboratory, laboratory-supplied sample containers (lid and bottle) are labeled with a number (#1-12) in black permanent felt pen (*e.g.*, Sharpie) in the order that the samples were collected. A temperature control sample is collected first and is labeled “TC” (Section 3.8), followed by sample #1. The corresponding site number for each sample is recorded on a DOH chain of custody sheet (Section 4.2.2).

When a site is sampled more than once during a day, the sample identifier is the sample site number followed by a letter starting with the letter “A” moving sequentially through the alphabet for each subsequent sample collected at that site on that day. This facilitates identification of samples collected at different times throughout a day.

When a sterile blank transfer is conducted in the field (Section 5.1), the sample is labeled as if it were a duplicate sample for the site at which the transfer is conducted. Regardless of whether a bacteria sample is collected at the actual site, the sterile blank is labeled with the sample site number followed by the letter “A.”

2.3 Representative Location

Bacteria samples are collected at a representative location. The following are considered when selecting a representative location at the sampling site:

1. Avoid areas along margins, where debris accumulates, and other areas at the sample site that are not characteristic of the waterbody.
2. Select an area that minimizes disturbance to the waterbody (Section 2.4).
3. For wading sites, unless safety precludes wading into the water, avoid collecting samples along the shoreline where waves are breaking and washing across the beach.

- Sample seaward of debris and seaweed generally found in the water close to the shoreline.
 - If samples are collected from within the wave or debris zone, assign a data qualifier and the reason in Water Database or on field datasheet. See Quality Management Plan (QMP) (LWRD 2018d) and Water Database User Guide (LWRD 2018e) for details.
 - Avoid areas of entrained air in the wave-wash zone.
4. If a representative location cannot be found, use professional judgment to determine whether the site should be sampled.
- If the site is not sampled due to inability to find a representative location, note this in Water Database or on field datasheet.
 - If the site is sampled, assign data qualifier due to non-representative sampling location in Water Database or on field datasheet (see QMP and Water Database User Guide for details).
5. If a site is sampled, and the site is recognized as non-representative after the sample is collected:
- Consider discarding the previously collected sample. If so, record actions in Water Database or on field datasheet with reason discarded and place a large “X” on the sample bottle label and lid.
 - If samples collected from non-representative location are retained for analysis, record the sample and result with qualifier code and the reason why in Water Database or on field datasheet.

2.4 Site Disturbance

Bacteria samples are collected first, prior to the collection of any other samples or measurement of *in-situ* water quality parameters. The sample site is examined to determine that it was not disturbed by the sampler prior to bacteria sample collection. A site is disturbed if sediments or other materials (*e.g.*, plants, benthic algae) settled at the bed of the waterbody are suspended into the water column, or debris falls into the water at the sample site. Fecal coliform bacteria in bottom sediments can remain viable for many weeks. Disturbing sediments can re-suspend these bacteria and result in temporary uncharacteristically high bacteria test results. Strategies for avoiding site disturbance include:

- Avoiding walking in or near the edge of the waterbody
- If wading into the waterbody is required, approach the sample site from the downstream/down-gradient side
- Using a wand to collect samples

Occasionally, it will be necessary to collect a second bacteria sample (*e.g.*, if the first attempt at sampling is unsuccessful). If the site was not disturbed by the first attempt at sample collection, the second sample attempt can follow immediately after the first with a new, sterile bottle. If

the area appears disrupted by the first sample collection attempt, consider the following when attempting to re-sample:

1. Subsequent samples should be at a different, but representative location at the sample site.
2. If overall conditions at the sample site are stable, the disturbance will dissipate within a few minutes, and if water quality is not likely to change from when sampling started, the disturbed location may be re-sampled after the disturbance has passed. If this approach is taken, record in Water Database or on field datasheet.
3. If subsequent samples cannot be relocated or sampled at a later time, consider sampling in the disturbed (non-representative) location and assign a data qualifier noting the reason in Water Database or on field datasheet.
4. If subsequent samples cannot be relocated or sampled at a slightly later time, consider a complete re-sampling of the site at a later time in the sample run. If this approach is taken, record in Water Database or on field datasheet.

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3. SAMPLE COLLECTION

Bacteria (*i.e.*, fecal coliform, *Escherichia coli*, and *Enterococcus*) samples are collected at marine and freshwater surface water sites and occasionally from ground water sites. Bacteria samples are always collected prior to any other sample collection or measurement of *in situ* water quality parameters.

When arriving at a surface water sample site, determine where the bacteria sample will be collected based upon visual assessment of the waterbody and previous experience:

1. Determine the path of sample collection in the waterbody prior to physically collecting the sample. The sample is collected at a representative location (Section 2.3).
2. Minimize disturbance to waterbody (Section 2.4).
3. Use the blue sampling wand to collect the bacteria sample (Section 3.1) unless unsafe or impractical. If site conditions prevent sampling using the wand (*e.g.*, very shallow water or extremely high flows), the bacteria sample can be collected by hand (Section 3.2).
4. Sample upstream/up-gradient of any influences of the sampler.
 - When sampling from the boat, sample water from near the bow (front of the boat) while slowly moving forward over water that the boat has not previously come into contact with during the sample run.
 - Sample outside of the zone of influence of the sampler(s) if the sample site requires wading. Water and sediments can be entrained by the movement of the sampler.

Special instructions for collecting bacteria samples for the ZAPS Technologies Liquid Station Continuous Water Quality Monitoring Project and from groundwater sites are provided in separate sections below.

3.1 Sample Collection With a Sampling Wand

Use sample wand for surface water bacteria sample collection unless impractical or unsafe (Figure 3.1).

1. Do not rinse sample bottle unless specifically instructed to do so by the laboratory.
2. Label closed (never opened) and undamaged laboratory-supplied sample bottle as described in Section 2.2.2.
3. Attach capped sample bottle to wand. The top of the sample bottle should be several inches upstream/up-gradient of every part of the sample wand (*i.e.*, no sample water will touch any part of the wand before flowing into the sample bottle).
4. Open sample container. Do not place bottle or cap on ground and do not touch or allow any foreign materials to come into contact with bottle opening or threads, or the inside of the bottle cap.
5. Vertically dip the sample bottle, opening first, into the water column and in one motion, rotate the bottle in the direction of the current (upstream), so that trapped air can

escape as the bottle fills in an upstream/up-gradient arc.

- The bottle should be no deeper than approximately 6 inches below the water surface.
 - The water from the surface should not enter the bottle, unless low flows prevent sampling of water below the surface.
6. Ensure that bottle opening is always upstream of wand.
 7. Continue the motion until bottle is nearly full, then rotate the bottle to remove it vertically from the waterbody.
 8. If the sample was collected successfully (Section 3.6), cap bottle and place upright in cooler with ice. If bottle cannot be placed on ice immediately after collection, place bottle in sheltered area out of direct sunlight and place in cooler as soon as possible. Record sample collection in Water Database or on field datasheet and on chain of custody form.
 9. If the sample is not collected successfully, place an “X” on the bottle label and lid and collect another sample. Do not collect sample from waters that were disturbed during collection of the unsuccessful sample (Section 2.4).
 10. Always sample bacteria first, before collecting any other samples or measuring *in situ* water quality parameters.



Figure 3.1 Bacteria Sample Collection Using a Sampling Wand

3.2 Sample Collection by Hand (Without a Sampling Wand)

Bacteria samples can be collected by hand if use of the sampling wand is unsafe, unpractical, or would cause site disturbance. Extremely high flows, such as those out of a culvert during high-flow seasons, can cause the sample bottle to detach from the sample wand. Very low flows can result in the sample wand disturbing the bottom of the waterbody during sample collection. In

these cases, and when use of the sampling wand is unsafe, a bacteria sample can be collected by hand as follows:

1. Do not rinse sample bottle unless specifically instructed to do so by the laboratory.
2. Label closed (never opened) and undamaged laboratory-supplied sample bottle as described in Section 2.2.2.
3. Hold capped sample bottle near base with hand. The top of the sample bottle should be several inches upstream/up-gradient of the sampler's hand (*i.e.*, no sample water will touch the sampler's hand before flowing into the sample bottle).
4. Follow steps 5-10 in Section 3.1.

3.3 Sample Collection in Extremely Shallow Waters

If the water at a sample site is too shallow to fully immerse a sample bottle, document the field conditions in Water Database or on field datasheet and use one of the following approaches while minimizing disturbance of the bottom of the waterbody. Do not allow water that has potentially or actually contacted the bottle and/or that has flowed past the bottle opening to enter the bottle. When filling the bottle, be careful not to create a wave at the container opening that can bring water from downstream of the opening back to the opening, and also not to entrain bottom materials in the vicinity of the bottle opening.

- For still waters, fill the bottle carefully by moving it slowly, opening-first, into the waterbody and then slowly moving it so that the bottle fills. Only water that has not been disturbed by the sampling is collected. Disturbed water includes water that has come into contact with the sample bottle. Do not stop movement of the bottle during filling.
- For moving waters, fill the bottle by carefully placing the bottle, opening first and pointed upstream, into the waterbody and then lowering the bottom of the bottle so that the bottle can fill. Depending upon the flow, the bottle can remain stationary or be moved upstream.

3.4 ZAPS Technologies Liquid Station Sampling

Bacteria samples collected from the ZAPS Technologies Liquid Station are collected from the sample spigot at the bottom of the ZAPS unit. The spigot is opened and flushed for approximately 30 seconds prior to sample collection to allow the lines to be cleared of sediment and other debris. Flush water is drained to the ground.

3.5 Groundwater Sampling

Occasionally, collection of a groundwater bacteria sample from a domestic or tribal supply well may be required. The bacteria sample must be collected prior to measurement of well water level. The bacteria sample may be collected before or after other water quality measurements, but must occur after the source has been flushed for three minutes.

1. Flush source from designated tap:
 - For active wells, adequate flushing is determined by stabilization of the temperature or by flushing the well for three minutes, whichever is longer.
 - For wells not pumped regularly, it is recommended that at least three casing volumes be pumped prior to sample collection.
 - Where well production is inadequate to flush the source as described above, flush source as much as possible and note sampling conditions in Water Database or on field datasheet. Assign data qualifier due to potentially incomplete flushing (see QMP and Water Database User Guide for details).
 - Do not collect the bacteria sample through a garden hose. If a hose was used for flushing the source, remove the hose.
2. Sterilize and re-flush:
 - Sterilize tap with bleach and/or propane torch.
 - Flush source again for at least one minute or as long as necessary to remove bleach. Do not reattach hose for flushing. Wait several minutes and, without touching tap or causing splashing of the tap, collect some water and determine if there is a bleach odor by wafting. Do not place nose above the bottle and sniff. Place cupped hand over the bottle and wave the air from the bottle toward the nose so as not to contaminate sample.
 - If no odor is present, allow tap to flush for one more minute before collecting sample.
 - If odor is present, flush again and repeat step 2.b.
3. Collect bacteria sample:
 - Label closed (never opened) and undamaged laboratory-supplied sample containers as described in Section 2.2.2.
 - Open bacteria sample container. Do not place bottle or cap on ground and do not touch or allow any foreign materials to come into contact with bottle opening or threads, or the inside of the bottle cap.
 - Fill bottle to nearly full. If sample is collected successfully, cap bottle and place upright in cooler with ice. If bottle cannot be placed on ice immediately after collection, place bottle in sheltered area out of direct sunlight and place in cooler as soon as possible. Record sample collection in Water Database or on field datasheet.
 - If sample is not collected successfully, place an "X" on the bottle label and lid and collect another sample. Record sample collection in Water Database or on field datasheet.

3.6 Successful Sample Collection

Bacteria samples must be collected successfully if they are to be delivered to the laboratory for analysis. Successful sampling means:

- The sample is representative
- The sample site was not disturbed prior to sample collection
- Laboratory instructions are followed
- The bottle is not contaminated by contact with sampler's hands or foreign materials such as plants and substrate of waterbody
- The cap is not contaminated by contact with sampler's hands or foreign materials

In cases where conditions preclude successful sampling (*e.g.*, the channel bottom was disturbed and a second sample could not be collected from a representative location or at a later time) and bacteria sample analysis is desired, a data qualifier must be assigned to the sample result in the Water Database due to the conditions of the sampling. See Section 2.4 for details on sampling a disturbed site. See the QMP and Water Database User Guide for details on assigning data qualifiers and entering comments into Water Database.

3.7 Storage of Samples

After sample collection, sample bottles are stored upright in a cooler on ice with lids screwed on tightly (unless laboratory instructions indicate otherwise) and packed to avoid breaking prior to delivery to the laboratory.

3.8 Additional Details for DOH Samples

Note that the first sample collected for DOH/NSSP sample runs (*e.g.*, Portage Bay and Lummi Bay DOH Support) is a temperature control sample. This sample bottle is not included on the chain of custody form and the sample bottle and lid are labeled "TC" for temperature control.

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4. LABORATORY ANALYSIS

Details on laboratory requirements and specific bacteria enumerated depend on the project, and are outlined in the relevant project QAPP. Laboratories used for bacteria analysis must be certified by Washington State. As of the writing of this SOP, the independent LNR contracted laboratory for most surface water and groundwater bacteria analysis is Edge Analytical, Inc. located in Bellingham, WA. Marine bacteria samples that are collected in partnership with the Department of Health (DOH) for the NSSP are analyzed by DOH Public Health Laboratory located in Shoreline, WA.

4.1 Holding Time and Laboratories

Bacteria samples collected by LWRD staff are sent to two laboratories: Edge and the DOH Public Health Laboratory, depending on the project. Table 4.1 lists the methods, sample requirements, upper and lower detection limits, and maximum holding times for the bacteria samples analyzed by these laboratories.

Table 4.1 Methods for Laboratory Analysis of Bacteria

	DOH Public Health Laboratory	Edge Analytical
Bacteria Tested	Fecal Coliform	Fecal Coliform, <i>Escherichia coli</i> , Enterococcus
Method	American Public Health Association A-1 Modified (5 tube, 3 dilution) method	Fecal Coliform: SM 9222D <i>E. coli</i> : SM 9222G Enterococcus: Enterolert
Sample Container	125 ml, sealed, sterile, plastic bottle with screw top. Provided by DOH.	250 ml, sealed (with autoclave tape), sterile, plastic bottle with screw top. Provided by Edge.
Sample Preservation	Ice (samples must remain within 0-10°C)	Ice (samples must remain within 0-10°C)
Maximum Holding Time	30 hours Note: holding time includes transportation time to the laboratory in Shoreline, WA.	8 hours Note: samples must be delivered to the laboratory within 6 hours of sampling to allow for processing within maximum holding time.

Table 4.1 Methods for Laboratory Analysis of Bacteria

	DOH Public Health Laboratory	Edge Analytical
Lower Detection Limit	1.8 fecal coliform CFU/100ml	Detection limits depend on sample filtration. Typical lower detection limits: 2 fecal coliform CFU/100 ml 2 E. coli CFU/100 ml 10 enterococci MPN/100 ml
Upper Detection Limit	N/A	Detection limits depend on sample filtration. Typical upper detection limits: 6,000 fecal coliform CFU/100 ml 6,000 E. coli CFU/100 ml 2,000 enterococci MPN/100 ml
Laboratory Address	Office of Public Health Laboratories Environmental Laboratory Sciences 1610 NE 150th Street Shoreline, WA 98155-9701	Edge Analytical 805 W. Orchard Drive, Suite 4 Bellingham, WA 98225

Bacteria samples are delivered to the laboratory within the holding time specified by the laboratory. On rare occasions bacteria samples may be delivered to Edge after more than 6 hours have elapsed, however samples are always delivered before 24 hours elapse. If samples are not delivered to the laboratory within the specified holding time, assign data qualifier and indicate the additional elapsed time with the reason why in Water Database (see QMP and Water Database User Guide for details).

4.2 Transportation to Laboratory and Chain of Custody

Bacteria samples are transported to the laboratory immediately after completion of sample collection. Samples are stored on ice until custody is transferred to laboratory staff. Detailed transportation and chain of custody information for delivery to the two laboratories (Edge and DOH Public Health Laboratory) are provided below.

4.2.1 Edge Analytical

Bacteria samples are stored in a cooler on ice during collection and transportation to the laboratory. Details regarding sample drop-off times are available directly from the contracted laboratory and in the Field Reference Manual (LWRD 2015b). Samples and chain of custody

form (see Appendix A) are transferred to the laboratory staff. The chain of custody form should be completed to include the Lummi Indian Business Council's (LIBC) contact information, a contact name in the LWRD, the project name (typically the run name), sample identification (if different from the location), location (site ID), matrix (SW for surface water), designate grab sample, date and time of sample collection (in 24-hour format), type of laboratory analysis requested (fecal coliform, *E. coli*, enterococcus), and number of containers. Laboratory staff will check the temperature of the samples and provide a LWRD staff a copy of the chain of custody form.

4.2.2 DOH Public Health Laboratory

Bacteria samples collected by LWRD to assist the DOH in implementing the Nssp on the Reservation are shipped via Greyhound to the DOH Public Health Laboratory in Shoreline, WA.

Bacteria samples are stored in a cooler on ice during the field run. Upon return to the lab, the samples are packed in a DOH-provided polystyrene ice chest so that all bottles are upright and the temperature control (labeled "TC") is in the center of the samples in the ice chest. All samples should be in contact with a reusable icepack, the ice chest packed with newspaper to prevent samples from shifting, and sealed completely with packing tape. Two or three reusable ice packs are used depending on season, for winter and summer respectively.

Each sample bottle is labeled with a number and is properly recorded with corresponding site number on the DOH chain of custody form (see Appendix B). The DOH chain of custody form is filled out as follows:

- Samplers – Lummi Natural Resources
- Area – location of the sample collection (either Lummi Bay or Portage Bay)
- Monitoring Program – regulatory
- Monitoring Agency – check "Other"
- Tide Information – tide location, high and low tide information to be completed in the relevant sample area
- Sam column – sample number written on the bacteria bottle/cap (#1-12)
- Sta column – station/sample site identification (*e.g.*, for DH044, write 44)
- Time – use military time (*e.g.*, for 3:25pm, write 15:25)
- Tide column – F for flood (tide coming in), E for ebb (tide going out). Flood conditions are present through one-half hour after high tide, and ebb conditions through one-half hour after low tide.
- SWT – surface water temperature (rounded up or down to nearest whole number)
- SS – surface water salinity (rounded up or down to nearest whole number)
- If both Lummi Bay and Portage Bay are sampled on the same date, separate chain of custody forms are used. Numbering should continue from one run to the next (*i.e.*, numbering not re-started on second chain of custody form).

The completed chain of custody form is sealed inside a zip lock bag and placed on top of the sealed ice chest inside the DOH-provided shipping box. The shipping box is sealed with packing tape and labeled "STAT" in red sharpie so that it is easily located by Seattle bus depot staff.

DOH samples packed in the shipping box are delivered to the Greyhound bus terminal front desk in Fairhaven for transport to Seattle on the same day the samples were collected. A DOH-supplied pre-paid shipping form will provide the Greyhound bus terminal employee the information needed to process the shipment. Field staff will stay at the bus terminal to ensure the Greyhound bus driver retrieves the box from the office and loads it on the bus. Greyhound bus schedules are subject to change, and should be checked frequently. If possible, the samples should be dropped off 30 minutes before the bus is scheduled to depart. A DOH contracted courier will pick up the package from the Seattle bus station at 7am the following morning and transport the package to the DOH Public Health Laboratory for analysis.

During times of the year when samples cannot be shipped via Greyhound the same day of collection (*e.g.*, samples are collected late in the day), the samples are delivered by LWRD staff to the Public Health Laboratory by 7:30 am the following day.

4.3 Laboratory Analysis

Fecal coliform bacteria and *Escherichia coli* (*E. coli*) are enumerated from the same plate to provide information about the presence of other bacterial species (*i.e.*, *Klebsiella*) in fecal coliform counts and increase the comparability of the fecal coliform bacteria and *E. coli* results. Enterococcus bacteria are enumerated from the same sample bottle as fecal coliform bacteria and *E. coli* to provide information about enterococcus distributions. The Environmental Protection Agency (EPA) is advocating for the discontinuation of fecal coliform as a regulatory indicator bacteria. However, at this time all three types of bacteria will continue to be enumerated for samples analyzed by Edge because NSSP criteria are based on fecal coliform counts and there is no indication that this will change.

4.4 Results

Edge posts preliminary bacteria results online at www.EdgeAnalytical.com and emails final bacteria results to the Water Resources Specialist II and Water Resources Technician II within one week of sample delivery.

DOH Public Health Laboratory results are emailed to the Water Resources Specialist II and can be downloaded from the DOH File Transfer Protocol site online.

5. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Quality assurance/quality control activities include sterile techniques, field blanks, temperature control samples, holding times, chain of custody forms, and laboratory QA/QC procedures.

5.1 Duplicates

Duplicate bacteria samples are not regularly collected to quantify precision due to the inherent spatial and temporal variability associated with bacteria. Where duplicate bacteria samples are collected, it is to assess field variability rather than laboratory variability.

5.2 Sterile Techniques

Field staff will ensure that all bacteria samples are collected using sterile techniques. This includes inspecting the laboratory-provided bacteria sample bottles for contamination prior to use (Section 2.2.1) and proper handling of the sample bottle during bacteria sample collection (Section 3). In particular, proper handling includes ensuring that:

- The bottle is not contaminated by contact with sampler's hands or foreign materials such as plants and substrate of waterbody
- The cap is not contaminated by contact with sampler's hands or foreign materials

5.3 Sterile Blank

Once per quarter, a sterile blank QA/QC sample is supplied to Edge for analysis. The sterile blank verifies the ability of field personnel to collect, handle, and transport bacteria samples using sterile techniques (*i.e.*, without contaminating the sample). It also verifies the ability of the independently contracted laboratory to process and analyze the sample without contamination. The sterile blank sample is a blind sample to the analyst at the independently contracted laboratory, as it is marked as a regular sample.

Sterile blank results are logged in an Excel spreadsheet managed by the Water Resources Specialist II on the LIBC network.

5.4 Temperature Control

Temperature control samples are collected during sampling for DOH/NSSP in Lummi and Portage bays. A sample bottle is filled with sample water at the beginning of the sample run to serve as a temperature control for the remainder of the sample run and delivery to the DOH Public Health Laboratory. The temperature control sample is stored in a cooler on ice the same way as all other samples are stored throughout the sample run. The DOH Public Health Laboratory staff measure the temperature of the temperature control sample upon receipt of the samples to the laboratory.

5.5 Holding Times

Laboratory holding times are observed for all bacteria samples collected. Table 4.1 denotes the holding times for the two laboratories currently providing bacteria sample analysis for LNR.

5.6 Chain of Custody Form

Chain of custody forms are used to handle and track samples from field collection to delivery (by hand or shipped) to the laboratory. A chain of custody form is provided by the independently contracted laboratory (Appendix A). The form is filled out while the sampler is in possession of the samples either during the sample run as information is recorded in Water Database or on field datasheets, or at the laboratory prior to releasing the samples. When the samples are transferred to the laboratory, the sampler signs and dates the chain of custody form to release the samples to the laboratory, and a designated representative from the laboratory signs and dates the form upon receipt of the samples. Laboratory staff examine the temperature of the samples received, record the average temperature on the chain of custody form, and provide a copy of the form to the sampler. The number on the chain of custody form will follow the samples through analysis to final reporting.

A chain of custody form is also provided by DOH for samples collected for the NSSP and delivered to the Public Health Laboratory in Shoreline, WA. The DOH chain of custody form also serves as the sample datasheet where sample results are recorded by the laboratory (see Appendix B for an example of a completed DOH chain of custody datasheet, and Section 4.2.2 for instructions on filling out the form). The DOH chain of custody form is completed by the sampler, placed in a ziplock bag, and included inside the sample delivery box.

All chain of custody forms are saved in three-ring binders in the LWRD offices and electronically on LIBC servers.

5.7 Laboratory QA/QC

The independent contracted laboratory is responsible for maintaining data quality for laboratory-analyzed results. Quality assurance samples may include blanks, positive growth tests, and negative growth tests. Quality assurance practices will meet or exceed method and accreditation requirements as outlined in the laboratory QAPP or method SOP. A summary of laboratory QA/QC requirements are provided in the Quality Management Plan (QMP Appendix C).

Department of Health laboratory QA/QC activities are conducted as required by DOH, NSSP, and accrediting agencies as outlined in DOH laboratory QAPPs and SOPs.

6. ACRONYMS AND ABBREVIATIONS

DOH	Department of Health
EPA	Environmental Protection Agency
LIBC	Lummi Indian Business Council
LNR	Lummi Natural Resources
LWRD	Lummi Water Resources Division
NSSP	National Shellfish Sanitation Program
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QMP	Quality Management Plan
SOP	Standard Operating Procedure

7. REFERENCES

- Lummi Water Resources Division (LWRD). 2015a. Health and Safety Plan. Prepared for the Lummi Indian Business Council. April.
- LWRD. 2015b. Lummi Nation Water Quality Monitoring Program: Field Reference Manual. Internal Document. July.
- LWRD. 2018a. Quality Assurance Project Plan: Ambient Surface Water Quality Monitoring Project. Prepared for the Lummi Indian Business Council. Lummi Reservation, Washington. December.
- LWRD. 2018b. Quality Assurance Project Plan: Department of Health Support National Shellfish Sanitation Program (NSSP) Project. Prepared for the Lummi Indian Business Council. Lummi Reservation, Washington. December.
- LWRD. 2018c. Quality Assurance Project Plan: First Flush Monitoring Project. Prepared for the Lummi Indian Business Council. Lummi Reservation, Washington. December.
- LWRD. 2018d. Quality Management Plan for the Lummi Nation Water Quality Monitoring Program. Version 1.0. Prepared for the Lummi Indian Business Council. Lummi Reservation, Washington. December.
- LWRD. 2018e. Water Database User Guide. Prepared for the Lummi Indian Business Council. Lummi Reservation, Washington. December.

8. APPENDICES

Appendix A: Edge Analytical Chain of Custody Form

Appendix B: Department of Health Chain of Custody and Sample Result Form

Appendix A: Edge Analytical Chain of Custody Form

EDGE ANALYTICAL

1620 South Walnut St. Burlington, WA 98233
 Microbiology (888-725-1212)
 805 W. Orchard Dr. Suite 4 Bellingham, WA 98225
Portland Lab (503-682-7802)
 9150 SW Pioneer Ct. Suite V Wilsonville, OR 97070
Corvallis Lab (541-753-4946)
 540 SW 3rd St. Corvallis, OR 97333
Bend Lab (541-639-8425)
 20332 Empire Ave. Suite F4 Bend, OR 97703

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CHAIN OF CUSTODY / ANALYSIS REQUEST (PLEASE COMPLETE ALL APPLICABLE SHADED SECTIONS)

Report To: LIBC (LUM05) Billing Email: HannaW@lummi-nsn.gov
 Address: 2665 Kwina Road Bill To: LIBC
 City: Bellingham State: WA Zip: 98226 Address: 2665 Kwina Road
 Attn: Hanna Winter City: Bellingham State: WA Zip: 98226
 Phone: 360-312-2312 Fax: Phone: 360-312-2313 P.O.#: 1000458
 Report Email: HannaW@lummi-nsn.gov Card: VISA M/C Expires:
 Project Name: FPW Card#:

FOR LAB USE

REF# 18-2474

CHECK REGULATORY PROGRAM

Safe Drinking Water Act
 Clean Water Act
 RCRA / CERCLA
 Other

INSTRUCTIONS "PLEASE READ"

- Use one line per sample location.
- Be specific in test requests.
- List each metal individually.
- Check off analysis to be performed for each sample location.
- Enter number of containers.

Analysis Requested

Sample ID	Location	Sample Type (See Below)	Grab Composite	Date	Time	Turn Around Time Required			Fecal coliform sm9222D (no Rosolic acid)	E. coli sm9222G (same plate as FC)	Enterococcus (Enterolert)	Number Of Containers	Special Instruction/ Conditions on Receipt
						<input checked="" type="checkbox"/> Standard	<input type="checkbox"/> Half-Time (50% Surcharge)	<input type="checkbox"/> Quickest (100% Surcharge) Phone Call Req. Emergency (Phone Call Required)					
1	SW051	SW	Grab	7/6/18	11:15	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1	
2	SW008	SW	↓	↓	11:30	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1	
3	SW053	SW	↓	↓	12:00	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1	
4	SW118	SW	↓	↓	13:19	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1	
5						<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
6						<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
7						<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
8						<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
9						<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
10						<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		

Sampled By: Hanna Winter Phone: 360-312-2312 Fax: Email: HannaW@lummi-nsn.gov
 Sample Receipt requested (Must include FAX or Email) CC: shyanana.j@lummi-nsn.gov

* Sample Matrix: W - Water SW - Surface Water WW - Wastewater OL - Oil
 DW - Drinking Water GW - Ground Water S - Soil Other

Custody Seals Intact: Yes No N/A
 Sample Temp: 9.8 C Satisfactory
 Evidence Of Cooling:
 Samples Received Intact:
 Chain Of Custody & Labels Agree:

Relinquished By	Date	Time	Received By	Date	Time
	7/6/18	13:48	JLW	7/6/18	13:48

Figure 8.1 Example Chain of Custody Form for Bacteria Samples Submitted to Edge Analytical

Appendix B: DOH Chain of Custody and Sample Result Form

SHELLFISH BACTERIOLOGICAL WATER QUALITY SURVEY

Date: 1/19/2010 Samplers: Lummi Bay Monitoring Agency: State Other of 1 of 1

Area: Lummi Bay Monitoring Program: Regulatory Date & Time Rcvd: 1/20/10 Page: 1 of 1

Tide Location: Sandy Point High Tide: Time 12:11 Height 9.4' Temp Control: 4.7°C Other: MB

Run ID#: 23556 Low Tide: Time 8:01 Height -0.2' Temp Control: 4.7°C Other: MB

Sam	Sta	Time	Tide	SWT	SS	Station Description / Remarks / BIDN	Lab ID #	WA #	Fcoli	Init	Rept Date
1	43	10:50	FL	7	26		1600418	246742	2.0	MB	JAN 22
2	42	11:04	FL	8	29		1600419	-001	<1.8		
3	41	11:25	FL	8	29		1600420		<1.8		
4	40	11:33	FL	8	29		1600421		<1.8		
5	39	11:40	FL	8	29		1600422		<1.8		
6	38	11:46	FL	8	29		1600423		<1.8		
7	288	11:52	FL	7	29		1600424		4.0		
8	286	12:03	FL	7	29		1600425		2.0		JAN 22
9	285	12:10	FL	8	26		1600426		7.8		
10	207	12:22	FL	8	30		1600427		<1.8		
11	45	13:33	EB	7	28		1600428		7.8		
12	44	13:50	EB	6	28		1600429	-012	<1.8	MB	

PRELIMINARY FOR OFFICIAL USE

RECEIVED

FEB 01 2010

Department of Health
Office of Environmental Health & Safety

Edited By: _____ Date: _____ Entered By: _____ Date: _____ Proofed By: _____ Date: _____

1/22/10 *JR*

P:\James Files\DOH\SBWQ SURVEY FORM 2014.doc

Figure 8.2 Sample Chain of Custody and Sample Result Form for Bacteria Samples Submitted to the DOH Public Health Laboratory as Part of the NSSP