(11/14/2015, Note: This SOP will be updated by EPA Region 10 lab before MST sampling begins, November 2015.)

Quality Assurance Project Plan
for
Drayton Harbor, Microbial Source Tracking Pilot Study, Cain Creek Update
EPA Region 10, Washington Operations Office (WOO)

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1.0 Project Management

1.1 Distribution List

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1.2 Project Organization

(EPA QA/R-5 A4)

The following individuals are responsible for design and implementation of this project, and/or will be the primary data users and decision makers:

- **Stephanie Harris**, (360) 871-8710, EPA Region 10 Environmental Laboratory investigator, is responsible for assisting with the preparation of the quality assurance project plan (QAPP), analysis of samples, and preparation of the final report.

- **Suzanne Skadowski**, (360) 753-9469, EPA project manager, will serve as the primary point of contact for the project.

- **Debby Sargeant**, (360) 236-3320,

- **Julie Hirsch,** (360) 758-4046, Hirsch Consulting Services. Project Manager, Sample Analysis Lead, Data Analysis Lead and Monitoring Technician Lead. Will provide

- **Stephanie Bailey**, (360) 871-8739, EPA microbiologist, will assist with project planning and analysis.

- **Geoff Menzies,** (360) 384-9135. Puget Sound Restoration Fund, Project Coordinator. Will….

- **Roy Araki**, (206) 553-6395, EPA Quality Assurance (QA) Officer, is responsible for assisting the project manager and investigator in the development of the QAPP, subsequent revisions, and amendments.

- **Brandon Perkins,** (206) 553-6396, EPA Regional Sample Control Coordinator (RSCC) for the EPA Region 10 Technical Support Unit, will coordinate sample analyses performed by the Region 10 Laboratory and will provide sample numbers for samples that will be analyzed at the Region 10 Laboratory.

1.3 Problem Definition/Background

This QAPP is updated to distinguish human fecal input to Cain Creek, a tributary to Semiahmoo Bay which in 2010 was found likely to influence Drayton Harbor that is currently in the TMDL implementation process.

Cain Creek is an urban watershed that drains a large portion of the city of Blaine to Semiahmoo Bay. Since 1999, water quality monitoring data have shown that fecal bacteria levels exceed water quality standards and may impact shellfish harvest along Semiahmoo Bay shorelines in the US and Canada and

inside Drayton Harbor (HCS, 2010). Impaired water quality affects tribal, commercial and recreational shellfish harvest in Drayton Harbor and in Semiahmoo and Boundary bays. The Federal Clean Water Act requires that a Total Maximum Daily Load is developed for impaired water bodies that calculates the amount of a pollutant that a waterbody can receive and still safely meet water quality standards. In 2008, the Washington State Department of Ecology began the bacteria Total Maximum Daily Load Study (TMDL) of the Drayton Harbor watershed (including Cain Creek) to improve water quality for safe primary contact and shellfish harvest. The TMDL study (Ecology, May 2010) found that reductions of fecal bacteria up to 95% at Cain Creek are required to meet the water quality standard. Data collected by Ecology and by H.C.S indicate that bacteria levels were relatively low at the headwaters of Cain Creek but increased sharply downstream where the subsequent sampling station was located. Data were collected by Hirsch Consulting Services in 2009 for the Nooksack Indian Tribe (NIT). This TMDL support study found that upper watershed sites met water quality standards but downstream sites exceeded both fecal coliform thresholds with geometric means up to 15 times the allowable level of 50 CFU/100 mL (599 – 762 CFU/100 mL). DNA microbial source tracking data that were collected during the study identified human fecal markers more often in water samples collect from downstream sites that correlated with extremely high fecal coliform densities.
This current study establishes four sampling sites that segment this portion of Cain Creek and characterizes water quality to assist in identifying source(s) of fecal bacteria. Because Blaine Public Works has partnered to provide fecal coliform analysis for this study for the express purpose of further resolving human *Bacteroides* markers identified during the 2010 study, additional MST data will assist them in determining whether high FC could originate due to potential problems with sewage collection lines in proximity of sampling locations. Through a grant funded by the Whatcom Community Foundation and partnership with the City of Blaine, the Puget Sound Restoration Fund is collecting data that will assist the City of Blaine in reducing fecal bacteria discharged to Cain Creek while involving high school students in the process. An understanding of water quality issues in their community will help these students to present to their City Council, data and their recommendations for improving water quality and making Cain Creek an asset for the community.

(EPA QA/R-5 A5)

1.3.1. Background

The health of Drayton Harbor has been a concern for nearly 20 years. Poor water quality and concerns about failing on-site sewage systems and livestock waste in the California and Dakota Creek subwatershed led to a downgrade of 500 acres of shellfish growing areas at the south end of the harbor in 1988. Further shellfish downgrades occurred in 1995 and 1999, due to continually degrading water quality throughout the harbor. Due to progress made addressing sources of pollution, by 2004 DOH was able to reclassify portions of Drayton Harbor to “Conditionally Approved” with closures occurring after large rainfall events. In spite of recent successes, harvesting is still Prohibited on several hundred acres of important tribal, commercial, and recreational shellfish beds. In addition, trends of high fecal coliform bacteria (FC) levels in Blaine Harbor marina continue.

In 2005, the Puget Sound Restoration Fund (PSRF) funded a literature review and case study analysis of microbial source tracking (MST) techniques appropriate for application to Drayton Harbor (Hirsch Consulting services [H.C.S.] 2005). This review was shared with local agencies to build support for an MST study in the Drayton Harbor watershed and to establish an approach for implementing corrective

actions based upon MST pilot study results. The review recommended a phased approach to MST beginning with a small- scale pilot study followed by a watershed-wide study based upon results of the pilot work. The recommended MST methods were *Bacteroides* host specific polymerase chain reaction (PCR) and ribotyping.

Whatcom County has begun a sampling project in tributaries of California Creek that lends itself to augmentation with MST techniques. The EPA Region 10 laboratory has proposed utilizing Drayton Harbor for a laboratory pilot study using the *Bacteroides* host specific (PCR) method. In addition, Whatcom County is in the process of initiating a pilot study to identify septic system input to California Creek sampling stations using sampling for optical brighteners

The PSRF has directed H.C.S. to prepare and implement a monitoring plan that incorporates the projects described above with DOH and PSRF marine sampling in Drayton Harbor, into an MST pilot study. The study is proposed to begin in the fall of 2006 to be completed in 2007.

Drayton Harbor Watershed and the California Creek Sub-basin

The Drayton Harbor watershed is located in the northwest corner of Whatcom County, Washington and straddles the international border with Canada. The watershed encompasses 35,102 acres including 256 acres in British Columbia. There are 129 miles of tributary streams contained within the Drayton Harbor Watershed. California and Dakota Creeks constitute the primary freshwater inputs to the Harbor draining over 90% of the watershed area (Peterson, 1995). The remaining portion of the watershed drains directly into Drayton Harbor. The mouth of Drayton Harbor lies just south of Semiahmoo Bay that is bisected by the international border with Canada. Figure 1 shows the boundaries of the Drayton Harbor watershed. Total annual rainfall recorded at Blaine averaged 41 inches per year between 1948-1998 with about 68% in the fall and winter ranging from 24.8 inches in 1952 to 52.7 inches recorded in 1997. The highest daily rainfall value was 3.4 inches in November 1955 (Determan, 1999).

Drayton Harbor is an enclosed marine embayment with a rich history as a shellfish resource (Figure 1). Shellfish harvest and growing has been restricted intermittently due to fecal pollution since 1952. In 1988 the Drayton Harbor watershed was designated the priority watershed in Whatcom County by the Puget Sound Water Quality Authority. Classification by the Washington State Department of Health (DOH) resulted in closure and restriction of portions of Drayton Harbor in January 1995. In September 1999, the remainder of the shellfish growing area was downgraded to Prohibited status.

In response to the January 1995 closure, Whatcom County established the Drayton Harbor Shellfish Protection District in July of 1995 and the Drayton Harbor Initial Closure Response Strategy was developed. In 2001 the Puget Sound Restoration Fund (PSRF) gained approval from DOH and Washington State Department of Natural Resources (DNR) to seed two acres in the central portion of Drayton Harbor with Pacific oysters as the Drayton Harbor Community Oyster Farm. The goal of the project was to involve community members in growing Drayton Harbor oysters for harvest in 2004. The oyster farm inspired efforts by various local agencies to control fecal pollution sources and water quality improvements were realized. In June 2004, DOH reopened 575 acres in the central portion of Drayton Harbor for conditional harvest based upon rainfall. Shellfish harvest was closed for five days immediately following more than ½ inch of rainfall in a 24-hour period (Lennartson, 2004). In February 2006, the thresholds for the conditional approval were adjusted resulting in a six-day closure following a

rainfall event of ¾ inches in a 24-hour period. Since the summer of 2004, 2000 bushels of oysters have been harvested most of which have been exported to China. Drayton Harbor is included on the state 303(d) list for fecal coliform bacteria and has been selected by the Washington State Department of Ecology (Ecology) for a Total Maximum Daily Load (TMDL) beginning in 2006 (Hood, 2006).

California Creek is one of two major streams in the Drayton Harbor watershed emptying into the harbor from the SE about ¾ mile south of the Dakota Creek entrance. The California Creek sub-basin is 13,762 acres which is about 40% of the total Drayton Harbor watershed area. Four stream systems and 5 major ditches drain into California Creek (Peterson 1995). The average mainstem flow estimated by the Northwest Indian College during bi-monthly sampling events from 2002 through spring 2005 was approximately 15.5 cubic feet per second (cfs) with a minimum of 3.52 cfs in August 2003, and a maximum of 43 cfs in November 2003 (Cochrane, 2005). The upper mainstem has been extensively channelized and impacted by agricultural activities and lacking vegetative cover. In 1991, a watershed characterization by the Puget Sound Cooperative River Basin Team found the lower mainstem was not as heavily impacted as were upper reaches, however, tributaries to the lower mainstem were heavily impacted (PSCRBT, 1991). In 2003 a wetland analysis estimated impervious surface at 5% in the California Creek sub-basin (Stanley, 2003). According to Stanley (2003), process changes to the hydrology and to the delivery of nutrients in the California Creek watershed have resulted in significant impacts to the water quality and habitat within Drayton Harbor.

California Creek soils are silt and clay estuarine deposits of the Sumas Stade glacial outwash yielding slow to moderate infiltration rates with fair bank stability (Matthews et. al, 1997). The high percentage of silt an clay particles in these soils results in low permeability with high run-off potential and pollutant transport. The California Creek terrace soils have a higher degree of saturation throughout the soil profile for a longer period of time than in the Dakota Creek sub-basin due to the presence of impermeable lacustrine and glaciomarine drift deposits (Stanley, 2003). A large peat deposit east of the town of Custer drains to California Creek and has been largely altered for pasture and cropland (PSCRBT, 1991).

Land Use

Land use in the Drayton Harbor watershed is diverse. Urban, residential, agriculture (commercial and hobby farms), surface mining, forestry, and marinas including industry are all identified as significant land uses in the watershed by the 1995 Drayton Harbor Management Plan (Peterson, 1995). The incorporated city of Blaine flanks the eastern shore of Drayton Harbor just south of the Canadian border. The Resort Semiahmoo and residential development borders the western shore of the harbor. California and Dakota Creeks discharge into the harbor from the southeast and east respectively. Custer is an unincorporated town located in the upper California Creek drainage. In 1991, the PSCRBT found that grazed pasture occupied 59% of land adjacent to stream corridors, with 30% forested and 8% in low density residential or recreational land use, with septic systems for sewage treatment. One hundred-thirty-four acres of Blaine's 3280 acre urban growth area encompasses 134 acres of wetland in the California Creek sub-basin. Figure 2 shows a land use map developed by Stanley (2003).

In 1998 Whatcom County passed the Agricultural Nutrient Management ordinance (WCC 16.28) regulating ground application of liquid manure. There are 18 commercial dairies in the Drayton Harbor

Watershed and a high concentration of hobby farms. The Whatcom Conservation District's 2005 Livestock Survey map is shown in Figure 3. The windshield survey identified 111 farms and a total of 4,276 animals with 4022 cattle (94%), 212 horses, 24 sheep, and a total of 18 swine, goat and alpaca (WCD, 2005). Locations of certified dairies compiled by the Whatcom Conservation District are shown in Figure 4.

On-site septic systems serve the sewage disposal needs for most of the California Creek basin. According to the Whatcom County Health Department there are 1,040 known OSS location within watershed boundaries as shown in Figure 5 (Whatcom County, 2006).

Beneficial Uses

Drayton Harbor was classified by the Department of Ecology as a Class A waterbody (WAC-173-201A-140) (State of Washington, 1997). Class A waters are considered excellent where water quality shall meet or exceed the requirements for all or substantially all uses. Under the 2003 standards revision Drayton Harbor is classified as excellent for aquatic uses, classified for shellfish harvest, primary contact recreation, wildlife habitat, harvesting, commerce/navigation, and aesthetics (WAC-173-201A-612, 2003). Tributaries of Drayton Harbor are given the same classification as the waterbody into which they merge unless they have their own designation, as Dakota Creek does. Characteristic uses include:

- Water supply (domestic, industrial and agricultural)
- Stock watering
- Fish (including salmonid) and shellfish spawning, rearing, migrating, and harvesting
- Wildlife habitat
- Recreation including primary contact (swimming), sport fishing, boating, and aesthetic enjoyment
- Commerce and navigation

Fecal coliform standards for Class A waters:

Freshwater	Geometric mean shall not exceed 100 colonies/100mL *and* not more than 10% of all samples shall exceed 200 colonies/100mL
Marine	Geometric mean shall not exceed 14 colonies/100mL *and* not more than 10% of all samples shall exceed 43 colonies/100mL. This is also the National Shellfish Sanitation Program (NSSP) criteria for approved shellfish growing waters except that NSSP uses the estimated 90th percentile calculation (NSSP, 1997).

Historically, Drayton Harbor has been a significant shellfish resource for several user groups. Environmental conditions were ideal for the harvest and farming of several species of clams and oysters. The Lummi Nation and the Nooksack Tribe hold ceremonial and subsistence rights to shellfish harvesting in the Harbor. Commercial shellfish growing began in 1905 when the Drayton Harbor Oyster

Company seeded 500 acres in the southern portion of Drayton Harbor. Neptune Aquafarms grew shellfish for commercial markets on 400 acres in the 1970s and 1980s until their assets were purchased by the Drayton Harbor Oyster Company in 1992 (Menzies, 1999). Commercial shellfish harvest by Drayton Harbor Oyster Company continued until 1997 when the shellfish growing area was downgraded. In past years, Semiahmoo Spit and the west side of Drayton harbor have produced 10-20% of the Lummi Nation shellfish harvest (Cochrane, 1999). Recreational harvest of shellfish has been popular at public and private beaches. Recreational harvest includes hard-shell clams, butter clams, and Dungeness crab.

Drayton Harbor watershed provides groundwater for the City of Blaine's public drinking water supply, small community water supplies, and individual wells. The watershed provides habitat for a variety of marine and freshwater fishes. Drayton Harbor and its tributaries, including ditches, support migration, rearing, and spawning of anadramous salmonids. Drayton Harbor watershed also supports spawning and rearing of native resident salmonids. The predominant species are coho and chum salmon, steelhead, and cutthroat trout. Chinook salmon also utilize the Drayton Harbor watershed (Peterson, 1995). The native spring chinook is listed as threatened under the federal Endangered Species Act. Forage fish (e.g. Pacific herring, sand lance, and surf smelt provide an important food source for salmonids and many marine birds and mammals in Drayton Harbor. The intertidal shoreline of Drayton Harbor and subtidal areas support extensive eelgrass beds that are sensitive spawning habitat for forage fish. Out migrating juvenile salmon and Dungeness crab also utilize these nearshore areas (PSCRBT, 1991). While California Creek is important from the fisheries standpoint, production is limited by stream channelization and ditching (Peterson, 1995).

Drayton Harbor is a popular destination for contact recreation, sport fishing, boating, and shellfish harvest. The Resort Semiahmoo and housing development is a destination location on Semiahmoo Spit offering proximity to a county park, beaches, recreational shellfish beds, and a 300-slip marina for pleasure craft. The Port of Bellingham operates a 681slip marina for commercial and pleasure craft at the northeastern side of the mouth of Drayton Harbor.

Potential Pollution Sources

Pollution sources identified by DOH in the 2004 sanitary survey (Lennartson, 2004) with the potential to impact affected shellfish growing areas include:

- Agricultural discharges: deficient manure management practices on farms located in the Dakota and California Creek watersheds;
- Port of Bellingham Marina: boat wastes from commercial and recreational watercraft; also, feces from large bird populations (gulls, cormorants, pigeons, ducks, etc.) on the rip-rap breakwater and the various building roofs and docks;
- Semiahmoo Marina: boat wastes from recreational watercraft and bird populations within the marina; also, feces from concentrations of seals which haul out on the floating breakwater surrounding the marina;
- Blaine sewage collection system: cracks or breaks in pipes, manholes, or lift stations located along Marina Drive; and, the jointed submarine force main which transports untreated sewage under the harbor mouth;
- Blaine sewage treatment plant (STP): discharges of partially treated effluent from the STP into Semiahmoo Bay;

- Seafood processors: discharge of process wastewater from these facilities into the area around sampling station 15;
- Stormwater discharges: several discharge points along the shoreline potentially transport pollution to the harbor from a variety of sources;
- Individual on-site septic systems (OSS): failing OSS situated along the creeks and the eastern and southern shoreline of Drayton Harbor.

The primary sources for potential fecal pollution in the California Creek sub-basin include agricultural, hobby farm manure management, stormwater runoff, failing OSS, and wildlife.

Existing Water Quality Monitoring Data

The DOH has been the primary source of fecal coliform data for shellfish growing areas in Drayton Harbor. The DOH has monitored shellfish growing operations in Drayton Harbor since 1952 for the purpose of classifying shellfish beds for commercial harvest. Currently, the DOH samples Drayton Harbor six times each year as part of their systematic random sampling (SRS) program. These data were supplemented by bi-monthly sample collection by the PSRF in 2004/2005. Since June 2004, PSRF has conducted wet weather marine sampling to provide additional data for use in supporting an increase in the rainfall threshold that triggers growing area closure under the DOH conditional classification. Current DOH classification is shown in Figure 9 along with recent data. Of 12 sampling stations, five meet the NSSP standards for harvest under low rainfall conditions but not under high rainfall conditions (4 of these stations are classified for conditional harvest), 3 stations do not meet standards under either rainfall condition, and 3 stations meet standards under both conditions (Whatcom County, 2006).

The Northwest Indian College collects water quality data for freshwater tributaries to Drayton Harbor including 3 stations along the mainstem of California Creek that have been sampled since 1999 (Cochrane, 2005). NWIC station locations are shown in Figure 5. Trends for moving geometric means at all three stations from 1999 through 2005 show decreases in FC levels until 2003 when they began to rise to current levels as shown in Figure 7. Geometric means for the past two years range from 56 to 65 FC/100 mL meeting the Washington State Water Quality standard for freshwater FC geometric means of 100 FC/100mL. However all three stations exceeded the 10% of samples greater than 100 FC/100 mL allowed by the standard, from 31% at C1 to 37% at C3 (Table 2).

Table 1. NWIC California Creek FC data summary; 2003 through 2005.

Station	Geomean	%>100	Min	Max	n
C1	65	31	10	310	26
C2	58	32	10	890	25
C3	56	37	10	570	30

Past studies have documented violations of fecal coliform water quality criteria in freshwater tributaries and nearshore marine waters in Drayton Harbor (Cook, 1987; Dickes, 1992; Saban and Matthews, 1992; Cykler, *et al.*, 1995; Vasey Engineering, 1995 and Matthews *et al.*, 1997, PSRF 2002). The Saban and Matthews (1992) study sampled tributary sites throughout the California Creek sub-basin on two occasions. Matthews et. al. (1997) selected 3 sites identified as priority stations from Saban and Matthews (1992) for a 1 year monthly sampling project. Cycler *et al.*, (1995) sampled one site on the mainstem of California Creek. In 2003, a watershed analysis of California and Dakota Creek sub-basins sampled California Creek and its tributaries at 13 locations for nitrate, dissolved oxygen, and conductivity to identify areas altered by land uses (Stanley, 2003).

In April 2006 the California Creek Tributary Monitoring Project (CCTMP) began sampling for fecal coliform bacteria at 10 sites representing distinct sub-basins of California Creek. The project includes a total of 10 sampling events with 4 quarterly random sampling events and 6 rainfall related events (H.C.S., 2006). Data for the 5 completed sampling events are presented in Table 2. Data reported for 5 sampling events indicates that 6 of the 10 stations failed the former Class A state freshwater fecal coliform geometric mean standard and they all failed the second part of the standard. (The standard was a geometric mean of no more than 100FC/100mL and no more than 10% of samples above 200 FC/100mL). Fecal coliform bacteria loading generally followed discharge patterns. Maximum FC counts of 1000 FC/100mL or above occurred at 6 out of 10 stations. The FC peaks occurred primarily after storm events in June and September (H.C.S. 2006a).

Table 2. California Creek fecal coliform summary data ranked in descending order by loading. (Bold indicates violation of former Class A geometric mean standard of 100FC/100mL.)

Station	Mean load FC/day (using FC log 10)	Mean Discharge (cfs)	Geometric Mean	Geometric Mean*	%>43	%>100	Min (FC/100 mL)	Max (FC/100 mL)	n
CA6	1.63E+08	3.86	**108**		80	60	23.0	440.0	5
CA15	4.50E+07	1.33	53	56	60	60	9	150	5**
CA3a	2.55E+07	1.10	46	92	80	60	3	540	5**
CA3	2.48E+07	0.72	53		33	33	6	1100	3
CA16	1.42E+07	0.26	**413**		100	80	380	1200	5
CA14	1.23E+07	0.20	**566**	**494**	100	100	140	1700	4**
CA4	1.00E+07	0.41	**141**		75	50	30	1000	4
CA8	3.90E+06	0.05	**230**	**165**	80	60	13	2200	5***
CA2	4.06E+06	0.09	77		100	33	48	160	3
CA13a	4.05E+06	0.12	**136**	**301**	80	80	20	1900	4**

* Geometric mean includes standing water or low flow samples (insufficient for discharge measurements) in July and September.
** Includes standing water/low flow sample in July and September no discharge or loading values.

1.3.2 Objectives and Goals

The health of Drayton Harbor has been a concern for nearly 20 years. Poor water quality and concerns about failing on-site sewage systems and livestock waste in the California and Dakota Creek subwatersheds led to a downgrade of 500 acres of shellfish growing areas at the south end of the harbor in 1988. Further shellfish downgrades occurred in 1995 and 1999, due to continually degrading water quality throughout the harbor. Due to progress made addressing sources of pollution, by 2004 DOH was able to reclassify portions of Drayton Harbor to "Conditionally Approved" with closures occurring after rainfall events of 0.5 inches in a 24-hour period. The conditional approval was further adjusted in 2006 to require closures after rainfall events of 0.75 inches in a 24-hours. In spite of recent successes, harvesting is still Prohibited on several hundred acres of important tribal, commercial, and recreational shellfish beds. Throughout the watershed, bacteria are still carried into the harbor during major rain events. The frequency of temporary closures due to rainfall events makes commercial harvest of shellfish currently impractical. The community is now turning its attention to the upper watershed, where on-site sewage systems and livestock waste from hobby farms are likely sources of bacteria. A particular emphasis has been placed on the California Creek subwatershed, where patterns of deteriorating water quality have been observed and the locations and densities of on-site sewage systems and hobby farms have been characterized. In addition, trends of high FC levels in Blaine Harbor marina continue.

In 2005, PSRF funded a literature review and case study analysis of MST techniques appropriate for application to Drayton Harbor (H.C.S., 2005). The review recommended a phased approach to MST in the Drayton Harbor watershed beginning with a small- scale pilot study followed by a watershed-wide study based upon results of the pilot work. A microbial source tracking project that can identify the presence of human, bovine, or equine contributions will assist in targeting solutions to improve water quality in the creeks and harbor.

This project serves to implement recommendations made in the PSRF MST review to use *Bacteroides* host specific polymerase chain reaction (PCR) and ribotyping to accomplish the following objectives at

selected California Creek sampling stations and at selected stations in Drayton Harbor and Blaine Harbor:

1) The primary objective of the study is identification of human sources at all selected sites during a wet season.

2) The secondary objective is to distinguish bovine and equine fecal pollutions sources at California Creek sites during a wet season.

1.4 Project/Task Description and Schedule

(EPA QA/R-5 A6)

This QAPP provides the supportive information used in developing the plan for collection and analysis of water samples from Drayton Harbor and California Creek and associated tributaries. The basic field and analytical tasks required to achieve the objectives of this project are listed below:

1) Collect grab samples of water from designated sites within these three waterbodies,
2) Analyze the samples for the presence/enumeration of fecal coliform, *E. coli* and *enterococci*;
3) Analyze the samples for the presence of species-specific *Bacteriodes* markers using polymerase chain reaction (PCR) technology.

The quality assurance (QA) requirements described in this document are critical to the success of this project and are derived from EPA QA/R-5 EPA Requirements for Quality Assurance Project Plans (March 2001).

Table 3 includes a schedule for conducting tasks related to this project. It is a guideline only as it is possible that unforeseen circumstances and conditions will require adjustment to some or all of the following proposed dates.

Table 3 – Schedule of Tasks

Project Schedule
This study will begin in November 2006 with completion before December 2007. The project timeline is shown in Table 3.

Table 3. Timeline for the California Creek/Drayton Harbor MST Pilot Study

Task	**2006**			**2007**											
	O	N	D	J	F	M	A	My	Jn	Jly	Ag	S	O	N	D
Monitoring Plan	x	x													
Freshwater sampling		x	x	x	x	x	x								
Marine Sampling		x	x	x	x	x	x	x							
Lab analysis									x	x					
Reporting											x	x	x		
Outreach														x	x

1.5 Data Quality Objectives and Criteria

Data from this phase of the project will be used as background to develop further data quality objectives for this project.

1.5.1 Objectives and Project Decisions

The primary data quality objective for the Drayton Harbor microbial source tracking (MST) study is to characterize the source(s) of contamination within the targeted water bodies to the following level of differentiation: human source, ruminant source, and/or non-human/non-ruminant source.

1.6 Special Training and Certification

The Region 10 Environmental Laboratory staff has completed the required annual 8-hour health and safety training. The Region 10 Laboratory is accredited by the National Environmental Laboratory Accreditation Council. The Region 10 Environmental Laboratory analysts have the appropriate training to conduct both bacteriological and molecular analyses. The fecal coliform analyses will be performed by an FDA-approved State Laboratory, under FDA's shellfish program. The DOE, DOH and Squaxin Island Tribe currently employ sample collectors that have the training necessary to appropriately sample ambient water.

1.7 Documents and Records

1.7.1 QAPP Distribution

It will be the responsibility of the project manager to ensure that appropriate project personnel have the most current, approved version of the QAPP, including updates (see Section 1.1 Distribution List). The final version of the QAPP and any updates will be distributed in portable document file format.

1.7.2 Determination Levels

Only those water samples determined to contain more than 14 colony-forming units of *fecal coliform* per 100 mL (MPN) will undergo further analysis for *Bacteriodes* species. This level may be adjusted later in the project based on sample results. The filters from sites below the cut off point will be retained for future testing.

1.7.3 Measurement Performance Criteria/Acceptance Criteria

The measurement performance criteria/acceptance criteria for this project are discussed in Section 2.4, Quality Control. In general, if a sample, or associated controls, fall outside of the acceptance criteria, they are invalidated and either re-sampled or re-analyzed, as appropriate.

1.7.4 Field and Laboratory Documentation and Records

Laboratory documentation will include but is not limited to raw data, sample preparation and analysis logs, and results of calibration and quality control (QC) checks.

Field documentation will include but is not limited to field notes, photographs, and sample data and chain-of-custody forms.

1.7.5 Quarterly and/or Final Reports

Field and laboratory documentation will be kept in a case file and submitted to the State (DOE and DOH), PSRF and EPA Region 10 Office of Water and Watersheds with the final report. The EPA Region 10 Laboratory will archive the following documents: (1) signed hard copies of sampling and chain-of-custody records; and (2) electronic and hard copies of analytical data. The Laboratory will store all sample receipt, sample log-in, and Laboratory instrument documentation for a minimum of ten years.

2.0 Data Generation and Acquisition

The elements in Sections 2.1-2.10 ensure that appropriate methods for sampling, measurement and analysis, data collection, data handling, and quality control (QC) activities are employed and documented.

2.1 Sampling Design (Experimental Design)

Samplers will collect grab samples of approximately 500 ml of water at each designated site. The sample will be split in the field and half will be sent to the EPA Region 10 Laboratory, the other half to the Institute for Environmental Health (IEH) Laboratories. The measurement parameter of interest is microbial. The proposed sampling locations include the following (these station numbers have been assigned by the DOH, the PSRF, Whatcom County Public Works Department and Hirsch Consulting Services for purposes of water quality results associated with shellfish growing areas and to track potential sources of fecal coliform bacteria):

2.1.1 Sampling Locations

California Creek

Table 4. Freshwater MST sites (5 sites)

Sample Site Number	Lat (48.)/long(122.)		Ribotype	Rationale for Site Selection	Location and Description	Land Use
CA3a	92575	68805		Third highest loading of 10 sites in CCMP	Ham Rd. approximately 1800 ft S of intersection w/Birch Bay Lynden Rd. downstream (E.) side	hobby farm, dairy
CA4	92583	66031		Geometric mean (GM) 4th highest of 10 CCMP sites, 141 FC/100 ML, flow throughout dry season	ValleyView Rd, 400 m N of Arnie Rd., upstream (N.) side.	dairy, hobby farms
CA6	92082	65221	X	Highest loading of 10 sites in CCMP, flow throughout dry season	Arnie Rd. where stream crosses rd. on N side, upstream from staff gauge	hobby farm, forested, urban(high OSS)
CA15	90072	61526	X	Second highest loading of 10 sites in CCMP	Portal Way near Farris across rd. from Dbl. L Mobile Ranch, 20 ft from concrete pipe	dairy. Hobby, OSS
CA16	91727	64944		GM 2nd highest of 10 CCMP sites, 413 FC/100 ML,flow throughout dry season	Bruce Rd.spur near Arnie (dead end of Main St, Custer, 3112 Main)	high OSS (>10)

Table 5: Marine Sites (Drayton Harbor, Semiahmoo Bay, 8 sites)

Sample station	Coordinates		Ribotype	Rationale for Site Selection	Description
	Lat. º N	Long. º W			
12	48.98296	122.78213	X	Conditionally approved recreational and tribal shellfish growing area.	Drayton Harbor west side, near shore inside Semiahmoo Spit. At west end and in line with row of pilings.
4	48.98067	122.75789		Prime commercial shellfish growing area, conditionally approved	Drayton Harbor, south end of the commercial growing area.
6	48.98544	122.75964	X	Prime commercial shellfish growing area, conditionally approved	Drayton Harbor, north end of the commercial growing area.
8	48.99001	122.76305		Highest FC concentrations in Drayton Harbor, prohibited status	Drayton Harbor, 20 ft. W of Blaine Marina breakwater S.W. corner.
15	48.99185	122.76677	X	Highest FC concentrations in Drayton Harbor, prohibited status	Semiahmoo Bay, at Eastern entrance to Drayton Harbor, 10 ft N. of Marine Drive dock
313	48.9784	122.7886		Likely to be affected by California Creek outflow	Offshore of concrete slab of burned out house.
314	48.9701	122.7806		Likely to be affected by California Creek outflow	Offshore of road up hill to posh homes
315	48.9655	122.7678	X	Likely to be affected by California Creek outflow	Offshore of last house at start of spit.

able 5. Marine MST sites.

Field Procedures

Sample Custody and Documentation

Samples will be placed into ice chests upon collection. Samples will be shipped to EIH and to EPA Region 10 Laboratory delivered for analysis within 24 hours of sample collection. A chain-of-custody (COC) form will accompany all samples to the laboratory. A temperature control sample will be submitted to the laboratory with each cooler of samples. Samples will be accepted for analysis only if the temperature control is measured below 10°C at the time of sample submittal.

Laboratory Procedures

Analytical methods, expected range of results, and required detection limits are summarized in Table 5.

Table 6. Analytical Methods Summary

Parameter	Description	Method	Lab	Sample Container	Preservation	Holding Time	Precision/ Quantitation Limits
Fecal coliform bacteria	Membrane filtration	APHA, 9222D	IEH	PE, 125 mL, sterile	10 °C, dark	(max)24-hours	20% RSD*/ 1 cfu/100 mL
HSPCR	PCR-2 marker	MEL SOPs	EPA	PE, 250 mL, sterile	10 °C, dark	(max)24-hours	**/1 DNA strand
	PCR-3 marker marker	IEH SOPs	IEH	PE, 500 mL, sterile	10 °C, dark	(max)24-hours	place holder
Ribotyping	2-enzyme	IEH SOPs	IEH	PE, 100 mL, sterile	10 °C, dark	(max)24-hours	place holder

* RSD-Relative standard deviation, standard deviation divided by the mean
** Standard Accuracy and Precision for analysis by PCR is unknown at this time. Identification is not quantitative.

Fecal coliform bacteria

Analysis of water samples for fecal coliform bacteria will be utilized by the EPA Region 10 Laboratory to screen water samples for PCR analysis. Freshwater samples will be analyzed by Avocet Environmental Testing in Bellingham Washington as part of the CCTMP, using the membrane filtration method, Standard Methods 9222 (APHA, 1998). The laboratory is accredited by Ecology for fecal coliform bacteria analysis. Marine samples will be analyzed at the DOH laboratory in Seattle using the most probable number (MPN), five-tube fermentation (Standard Methods 9221 E(a) using A-1 Medium) technique. Samples will be analyzed in conformance with National Shellfish Sanitation Program (NSSP) guidelines (NSSP, 2003). Analysis of water samples for fecal coliform bacteria will be utilized by EIH for screening and colony isolation for ribotyping. Water and sewage grab samples will be processed for fecal coliforms using the membrane filtration method, Standard Methods 9222 (APHA, 1998).

PCR and Ribotyping

Bacteroides HSPCR is the primary DNA microbial source tracking method that will be utilized at each

selected sation. Ribotyping will be used for a subset of samples to corroborate PCR results and to further distinguish fecal sources. Water samples from the CCTP sites will be split for analysis by HSPCR at the IEH laboratory utilizing human, bovine, and equine primers and at the EPA Region 10 laboratory using human vs. ruminant primers. Marine samples will be analyzed by host specific PCR at the EPA's MEL where only human and ruminant primers are available. Methods at both laboratories were derived form the PCR method developed by Kate Field at Oregon State University.

The EPA Region 10 Laboratory will follow the method developed by Office of Research and Development the DNA extraction, polymerase chain reaction and gel electrophoresis methods utilized in this study as Standard Operating Procedures as referenced in the EPA QAPP for this study (MEL, 2006).(Quality Assurance Project Plan for Drayton Harbor Microbial Source Tracking Pilot Study EPA Region 10, Washington Operations Office (WOO). including:

- Sample Preparation
- Sample Filtration
- DNA Extraction
- PCR and Electrophoresis
- Gel Visualization

2.1.2 Sampling Schedule

4) Samples will be collected eight times at each site. The November 2006 through April 200 period has six sampling events scheduled. Proposed sampling dates for the project correspond with The Region 10 Laboratory must receive all samples no later than Wednesday of the sampling week, to allow for reading of the samples without staff overtime/compensatory time charges. Oakland Bay is less than a one hour drive from the EPA Region 10 Laboratory. For routine fresh water quality monitoring, DOE and Squaxin Island Tribe staff can typically collect samples in the morning and deliver them to the Region 10 Laboratory by noon the same day. Samples will be transported on ice within 30 hours of sampling to the DOH Laboratory in Seattle for fecal coliform enumeration.

2.2 Sampling Methods

2.2.1 Sample Collection and Handling

The samples will be collected following guidelines as cited in EPA-600/8-78-017, 1978, Microbiological Methods for Monitoring the Environment: Water and Wastes. Sampling methods may vary slightly between standard operating procedures used by samplers and different conditions encountered in the field. The following is general guidance for the samplers.

If samples are collected manually, rubber gloves should be worn and the use of safety glasses should be considered.

When the collection site is identified, the sampler should collect the sample as follows:

When dip samples are taken for analysis, the sampler should carefully remove the foil cap, ensuring that neither the inside of the sample bottle or the cap are touched. Hold the foil covered cap, do not set it down.

When possible, take the sample by holding the bottle near its base and plunge it neck downward, below the surface. Use an extension pole, if needed, to keep from walking into the stream and stirring up the sampling area. Turn the bottle until the neck points slightly upward and mouth is directed towards the current. If there is no current, create a current by pushing the bottle forward horizontally in a direction away from the hand or pole. For surface waters, samples should be collected far enough below the surface to avoid contamination with any surface scum or floating detritus, such as leaves or other debris.

After collection, carefully recap the sample bottle securely, leaving the foil cap in place. There should be a 1 inch head space in the neck of the bottle, to allow adequate mixing by the analyst. If, however, the sample container is overfilled, DO NOT pour out any excess sample. Place the cap securely on the sample bottle and return it to the analyst overfilled and a notation will be made in the analyst's report.

Water samples will be collected using either a sampling wand or hand dipping in midstream. Water sampling will be conducted in accordance with the water sampling protocol for coliform bacteria detailed in Standard methods 9060A and 9060B (APHA, 1998) and in the Washington State Department of Health Procedure #003 (DOH, 1996). All sample containers will be pre-sterilized and provided by IEH or EPA Region 10 laboratory. Samples for HSPCR will be collected in a 500ml sterile plastic bottles provided by EPA Region 10. Samples will be immediately split into 250 mL bottles provided by MEL. Both sets of samples will immediately be placed on ice in separate coolers. Samples for fecal coliform and subsequent ribotyping analysis at IEH will be collected in 120 mL sterile bottle provided by IEH. Standard sampling procedure for ribotype samples includes collection of 5 field replicate samples per sampling station per sampling event. Fecal coliform analysis is performed upon each of the 5 replicated samples. At least 2 *E. coli* isolates are utilized from each of the 5 samples for ribotyping analysis. The results for these fecal coliform analyses will be provided to the EPA Region 10 Laboratory within 72 hours.

Samples will be delivered to the IEH and MEL within 24 hours of sampling. Each bottle will be labeled with a site number prior to sampling. Site numbers will be recorded on write-in-the-rain field data sheets prior to sampling. Site numbers, date, and time sampled for each sample will be transcribed to the chain-of-custody sheet prior to submitting samples to the laboratory. The following information will be recorded on field data sheets.

- Sampler(s) name(s)
- Site numbers
- Field Replicate numbers
- Laboratory replicate numbers
- Date
- Time sample collected
- Additional observations
- Weather conditions

- Temperature control °C (measured upon arrival at laboratory)

A transfer blank will be created each day that samples are collected. The transfer blank is a bottle containing sterile water that is prepared at the laboratory. The purpose of the transfer blank is to check for potential contamination of samples during collection and transport. Halfway through the sampling for the day, the sampler will transfer the contents of the bottle containing sterile water into a sterile sample bottle, being careful not to contaminate the inside of the bottle or cap during transfer. The sampler will label this bottle with the date and time of transfer, name of collector, sample number and label the bottle as a 'Transfer Blank'. This transfer blank will serve as a blank for microbial and DNA extraction testing.

2.2.3 Sample Custody and Transport

A unique sample number assigned by the RSCC will identify each sample. Transporters will use EPA Region 10 chain-of-custody procedures and forms. Samples will be hand-delivered to the EPA Region 10 Laboratory. Samples will also be transported to the Avocet Environmental Testing, Inc. for fecal coliform enumeration.

2.2.4 Health and Safety

When working with potentially hazardous materials, investigators are to follow USEPA, Occupational Safety and Health Administration and site-specific health and safety procedures. Since these waterbodies may be contaminated with fresh fecal material, it is advised that samplers wear gloves and wash their hands prior to handling food or food products.

2.3 Analytical Methods

The EPA Office of Research and Development has developed the DNA extraction, polymerase chain reaction and gel electrophoresis methods utilized in this study as Standard Operating Procedures. The SOPs are in Appendix C-2 of this document.

2.4 Quality Control (QC)

The following QC activities will be performed by the laboratories performing analytical services in support of this project.

2.4.1 Samples Analyzed by the EPA Region 10 Laboratory

Polymerase Chain Reaction (PCR) Samples:

Filtration Controls: The purpose of filtration controls for PCR is to ensure that proper aseptic technique is used during sampling and laboratory filtration to avoid contamination.

Negative Controls: For each sampling batch (1-20 samples processed on the same day), one filtration negative control will be prepared using the transfer blank for that day. For each sampling batch, a second filtration negative control will also be prepared using 100 mL of ultra-pure water. Field

blanks monitor for the introduction of extraneous material into the samples during field sample handling, transport and storage. A positive result indicates the presence of contamination most likely due to poor aseptic technique in the field, contact with other samples or damaged storage containers. The method blank (filtration blank) is designed to screen for contamination throughout the entire MST process. False positive results indicate contamination at some point in the process. False positive results may not disqualify the data if a cause can be determined and if the cause is determined not to impact the remainder of the samples processed in that batch.

PCR Controls: The purpose of the PCR amplification controls is to ensure that the amplification system (the polymerase, master mix, templates etc) is functioning properly, cross contamination is absent and that a negative result in an unknown (sample) is actually negative for that particular primer. The following controls are assessed in each analysis (i.e. for each primer, which is the species specific DNA strand used for DNA replication).

1) One positive control, consisting of plasmid DNA containing the target sequence or the appropriate fecal DNA. This will serve as the reference sample.
2) One extraction negative control, made by following the extraction protocol with ultra pure water rather than a sample.
3) One PCR negative control, made by setting up PCRs with water instead of template.
4) A actual sample will not be considered negative until it has been subjected to replicate analyses using at least 5 different concentrations of the sample containing the purified DNA.

Results that are out of specification will be reviewed by the Technical Director for Microbiology and the Project Officer for a decision on whether the data should be included in the report. Method experts in EPA's Office of Research and Development (ORD) may also be consulted for advice on the quality of the data in these situations.

Replicate Analysis (Analyst and Method Precision):

Identical replicate analyses: Identical replicates (i.e. aliquots of the same sample) are assayed simultaneously using the same sample preparation, method and the same reagents. These replicates can serve two functions. They can preserve data (in case of replicate failure) and they can be used to monitor variability in the method and/or analyst's performance of the method. While replicate filters will be prepared for 100% of the field samples, at least 10 % of these filter replicates will be subjected to the remainder of the analyses (extraction, gene amplification, and gel electrophoresis) to access precision. EPA ORD's Microbial Source Tracking Guide Document (June 2005) does not contain a numerical standard to apply to precision data resulting from identical replicate analyses, however, the results for these identical replicates should be in agreement. If they are not in agreement, the overall results for that batch of samples will be evaluated for validity in discussions between the Technical Director for Microbiology, Project Officer and method experts from ORD.

Method Accuracy: Blind Sample Control:

PSRF or its designees will submit one water sample containing horse, cow, or human fecal material (on a rotating or intermittent schedule) for a 'blind' PCR analysis by the Region 10 Laboratory. The blind sample will be clearly identified as such (without identifying the source of the fecal material)

and will be submitted with each sample event batch or intermittently. The blind sample will be submitted for PCR analysis only. The results of these blind samples will be used to establish a base line expectation for the Laboratory. Currently, there is no established guideline for method accuracy using blind samples. If the method fails to correctly identify the type of fecal material in a blind sample, the overall results for that batch of samples will be evaluated for validity in discussions between the Technical Director for Microbiology, Project Officer and method experts from ORD.

Specificity:

Specificity is the ability of a given MST method to discriminate between various animal sources. Known animal sources are used to ensure that the primers work in a given geographic region. Availability of useful and applicable scat samples from the Drayton Harbor watershed is problematic, so regional samples (collected from the Puget Sound area) will be used to evaluate the specificity of the primers. Specificity testing can be used to provide a specific pattern-detectable percentage as follows:

$$\text{Specificity} = \frac{\text{Test Negatives}}{\text{Test Negatives + False Positives}} \times 100$$

Although there is currently no consensus, specificity values below 80 percent reflect questionable discriminatory power.

The following table summarizes the quality control for the methods to be carried out at the Region 10 Laboratory.

	Duplicates[1]; *Frequency* (corrective action/consequence of failure)	**Positive Control[2]; *Frequency* (corrective action/ consequence of failure)**	**Negative Control[2]; *Frequency* (corrective action/consequence of failure)**	**Blind Control[2]; *Frequency* (corrective action/consequence of failure)**
Polymerase Chain Reaction	Identical replicate analysis using the same sample preparation, method and reagents. *Replicate filters will be prepared for 100% of the field samples; at least 10 % of these filter replicates will be subjected to extraction, gene amplification, and gel electrophoresis* (may or may not disqualify data depending on consequences of cause)	Reference sample/positive control of plasmid DNA containing target sequence or the appropriate fecal DNA; *for each analysis/primer* (may or may not disqualify data depending on consequences of cause)	a) method/filtration control using transfer blank assessing entire process; *one/1-20 samples/day* (may or may not disqualify data depending on consequences of cause) b) filtration control using ultra-pure water; *one/1-20 samples/day* (may or may not disqualify data depending on consequences of cause) c) one extraction negative control using extraction protocol with ultra pure water; *for each analysis/prime* (may or may not disqualify data depending on consequences of cause) d) one PCR negative controls - PCRs with water instead of template; *for each analysis/primer*(may or may not disqualify data depending on consequences of cause)	PSRF water sample containing horse, cow, or human fecal material for a 'blind' PCR analysis *with each sample event batch or intermittently* (may or may not disqualify data depending on consequences of cause)

1 To measure precision
2 To measure accuracy
3 Per the SOP

2.4.2 Samples Analyzed by Avocet Environmental Testing Laboratory, Inc

Fecal Coliform Enumeration:

Fecal coliform will be analyzed at the Avocet Environmental Testing, Inc. laboratory in Bellingham using the most probable number (MPN), five-tube fermentation (Standard Methods 9221 E(a) using A-1 Medium) technique. Samples will be analyzed in conformance with National Shellfish Sanitation Program (NSSP) guidelines (NSSP, 2003).

Microbial Identification and Enumeration: The purpose of these controls is to ensure that the quality system associated with the filtration process for bacteria is within the criteria established below.

Comment [SH1]: What kind of QC and media will be used by Avocet??

Negative Controls: For each batch of prepared media, the appropriate negative culture control organism will be tested to ensure that the media demonstrates the correct reaction. These reactions are media-specific and are detailed in the specific method SOP. Media that does not meet these criteria will not be used in the analyses, and will be discarded. For each sampling batch (1 -20 samples processed on the same day), a filtration negative control will be prepared using sterile rinse buffer. If a positive result occurs, the samples for that batch will be invalidated and samples recollected. Each day of analysis, one negative culture control will be seeded and incubated in each operating water bath. Inappropriate results for the negative control will result in invalidation of all samples with which that negative control was incubated.

Positive Controls: For each batch of prepared media, the appropriate positive culture control organism will be tested to ensure that the media demonstrates the correct reaction. Media that does not meet these criteria will not be used in the analyses, and will be discarded. Each day of analysis, one positive culture control will be seeded and incubated in each operating water bath. Inappropriate results for the positive control will result in invalidation of all samples with which that positive control was incubated.

Confirmatory results: Tubes with turbidity and no gas will be subjected to a confirmatory step by transferring a small amount of the culture into EC broth with MUG (4 methyl-umbelliferyl-ß-D-glucuronide), incubating and observing for proper reaction.

2.5 Instrument/Equipment Testing, Inspection, and Maintenance

2.5.1 Field Measurement Instruments/Equipment

This phase of the project requires no field instruments.

2.5.2 Laboratory Analysis Instruments/Equipment

Laboratory instruments such as waterbaths, incubators, thermal cyclers and other equipment required by the applicable analytical methods will be maintained according to the manufacturers' instructions and the Laboratory SOPs. Records for equipment service shall be maintained by the Laboratory.

2.6 Instrument/Equipment Calibration and Frequency

2.6.1 Field Measurement Instruments/Equipment

No field instruments will be used during this phase of the project.

2.6.2 Laboratory Analysis Instruments/Equipment

Laboratory equipment (e.g. pH meter, waterbath, etc.) will be calibrated using the method and frequency specified in the Laboratory's SOPs. Records on calibration of laboratory equipment shall be maintained by the Laboratory.

2.7 Inspection/Acceptance Requirements for Supplies and Consumables

2.7.1 Field Sampling Supplies and Consumables

Consumable supplies used in the field will consist primarily of sterile plastic sample containers. The quality of consumable supplies such as plastic sample bottles used for this project should be documented by the supplier and certificates should be available on request. Bottles provided by the Region 10 Laboratory will be batch tested for sterility prior to sending them to the field for sample collection, as per SOP Mi_G004A. Bottles other than those prepared at the Region 10 Laboratory will not be acceptable for use during this project.

2.7.2 Laboratory Analyses Supplies and Consumables

The quality of chemicals, media and other supplies and consumables used in the Laboratory is dictated by the sensitivity and specificity of the analytical techniques being used. In the Region 10 Laboratory, chemicals, media, and other supplies and consumables are marked with the date received and the receiver's initials. In the event an expiration date has not been assigned by the manufacturer, the expiration date will be assigned by the receiver according to the Region 10 Laboratory's work instruction, "General Guidelines for Assigning Standard Expiration Dates." The quality of all laboratory supplies is documented by the supplier and the Region 10 Laboratory requests and keeps the vendor certificates on record per NELAC requirements.

2.8 Data Acquisition Requirements (Non-Direct Measurements)

Not applicable to this project**.**

2.9 Data Management

A field notebook, photos, and the Field Sample and Chain-of-Custody Data Sheets will be used to document the sampling activities. The Field Sample and Chain-of-Custody Data Sheets will have the following information: site name, sample number, date and time of each sample collection, and the sampler's name or initials and if possible, notes regarding human or animal activity in the area at the time of the sampling. In addition, the Laboratory will maintain a logbook that includes the above

information provided in the Field Sample and Chain-of-Custody Data Sheets, as well as time of analysis and analyst initials. Quality control results will be recorded on bench sheets. All data generated by Region 10 will be subject to a peer review then signed-off by the Microbiology Team Technical Director. Data entry staff will process and distribute all information mentioned above in accordance with the Laboratory's SOP. Logbooks, bench sheets and final reports will be stored on-site. All data generated during this project will be processed, stored, and distributed according to Laboratory SOPs.

3.0 Assessment and Oversight

3.1 Assessments/Oversight and Response Actions

Laboratories routinely perform performance checks using method-specific positive and negative controls, blind samples, etc. The positive controls, consisting of plasmid DNA containing the target sequence or the appropriate fecal DNA, will be provided by EPA's Office of Research and Development. Blind samples will be provided by the Squaxin Island Tribe. An internal assessment of the data and results is also routinely conducted by the supervisor and the Laboratory QA Coordinator.

A quality assurance assessment beyond routine QA procedures will be conducted during the course of this project. Given the short time frame, only one assessment is planned before completion of the project. The quality assurance assessment performed during this project will include the following:

1) Oversight of field sampling activities.
2) Oversight of sample handling and chain-of-custody procedures.
3) Laboratory inspection.

The Quality assurance assessment will be conducted by the EPA Region 10 Quality Assurance Officer or QA staff delegated by the Officer to conduct the assessment.

Corrective actions will be implemented in response to any QA results or detection of unacceptable data. These corrective actions will be developed in consultation with the Office of Research and Development, keeping the data user informed of any impacts on the data. If required, corrective actions will be documented in Appendix A-3.2, Corrective Action Form.

3.2 Reports to Management

If, for any reason, the schedules or procedures above cannot be followed, the project manager shall work with the appropriate parties to complete the Sample Alteration Form (SAF) found in Appendix A-3.1. The SAF will be reviewed and approved by the QA Officer and the Laboratory(ies) and/or samplers will be given a copy of the QA Officer-approved SAF for reference and for the project file.

A final report will be generated at the completion of the project. This report will include a discussion of the findings, interpretation of data and an executive summary. The report will be provided to all individuals listed in section 1.1 (Distribution List). In addition, short summary reports will be provided during the time of the study to keep those on the distribution list informed of the progress of the project. Difficulties that may result in delayed data and reporting will be communicated to those parties on the Distribution List.

4.0 Data Review and Usability

4.1 Data Review, Verification, and Validation Requirements

4.1.1 Data Verification/Peer Review

Region 10 data verification and peer review will be accomplished following the Laboratory's SOP Mi_D001A (Data Review). Data will be qualified as necessary to convey to the user any important information that needs to be considered in its use.

4.1.2 Data Validation

Data validation is an evaluation of the technical usability of the verified data with respect to the planned objectives of the project. This is accomplished by applying a defined set of performance criteria to the body of data in the evaluation process. Data validation for this project will be performed by the project manager.

4.2 Verification and Validation Methods

4.2.1 Data Verification

Data verification will include a review of the findings of all QA assessment activities including:

1) Field collection procedures
2) Sample labeling methods.
3) Chain-of-custody procedures.
4) Assessments of analytical data collection, recording, and reporting.

Verification of field collection procedures, sample labeling methods and chain of custody procedures are the responsibility of the Project Manager. Verification of the Region 10 analytical results is the responsibility of the Microbiology Technical Director, as required by the Laboratory's QA Manual. If any deviations are identified, the potential impact of those deviations on the reliability of the data will be assessed, and the information will be provided to the project manager through the QA Memo and appropriate flagging of the data.

4.2.2 Data Validation

Data validation will evaluate all individual samples collected and analyzed to determine if the results are within acceptable limits. Quantitative or qualitative limits of acceptability are defined for precision, accuracy, representativeness, comparability, and completeness.

1) Precision is defined as the agreement between a set of replicate measurements without assumption and knowledge of the true value. Agreement is expressed as either the relative percent difference (RPD) for duplicate measurements or the range and standard deviation for larger numbers of replicates. Data on precision are obtained by analyzing duplicate and replicate samples. RPD is not applicable to qualitative data.

2) Accuracy is a measure of the closeness of a sample analysis result to the "true" value. Accuracy will be determined primarily by an evaluation of the agreement between repeat analyses within the laboratory.

3) Representativeness is defined as the degree to which data accurately and precisely represents characteristics of a population, parameter variations at a sampling point, or an environmental condition. For this project, representativeness will be ensured by selection of sampling locations in accordance with the sampling design requirements in this QAPP.

4) Data are comparable if collection techniques, measurement procedures, methods, and reporting units are equivalent for the samples within a sample set. Comparable data for this project will be obtained by specifying standard units for physical measurements and standard procedures for sample collection, processing and analysis.

5) Data are complete when a prescribed percentage of the total intended measurements and samples are obtained. Analytical completeness is defined as the percentage of valid analytical results requested. For this project, acceptable completeness is > 90%.

4.3 Reconciliation with User Requirements

All data and related information obtained during the course of this project will be included in a data report. Presentations of data and data analysis may be made to relevant user groups upon request.

APPENDICES

Appendix A. Sampling Design and Data Quality Procedures

- A-1. Figures
- A-2. Tables
- A-3. Data Quality Forms

Appendix B. Field Documentation

- B-1. Equipment/Instrument Manual
- B-2. Standard Operating Procedures
- B-3. Field Data Forms

Appendix C. Laboratory Documentation

- C-1. QA Manual
- C-2. Standard Operating Procedures
- C-3. Data Report Forms

Appendix D. Data Evaluation

APPENDIX A. SAMPLING DESIGN AND DATA QUALITY PROCEDURES

Appendix A-1. FIGURES

Figure 1-1. Organization

Project Organization: Refer to section **1.2** for project participant roles and responsibilities.

Figure 2-1. Site Map with MST Sampling Locations

Comment [SH2]: Julie, can those maps that you had before be added to this section with the appropriate headers??

Figure 2-2. 2006 Drayton Harbor Shellfish Growing Area Classifications and DOH Sample Sites.

Appendix A-2. TABLES

Table A-1. Data Quality Objectives Summary

Analytical Group	Number of Samples	# of QA Samples: Reference Samples	Matrix	Method	Method Detection Limits	Accuracy	Precision (RPD)	Completeness	Volume, Container	Holding Time (days)
				Laboratory Measurements						
Fecal coliform	12 per sampling event	1 each batch	Water	Standard Methods 9221E(a)	1 CFU	+/- 10%	+/- 10%	> 90%	250 ml PP, sterile	6 hours
Filtration	12	1 each batch	Water	ORD prep by filtration	1 strand DNA	See Section 2.5.1	See Section 2.5.1	> 90 %	500 ml PP, sterile	30 hours
PCR	Varying depending on fecal coliform levels	1 each batch	Frozen prepared filter	*Bacteriodes* identification	1 strand DNA	See Section 2.5.1 [1]	See Section 2.5.1 [1]	> 90 %	500 ml PP, sterile	none

1 – Standard Accuracy and Precision for analysis by PCR is unknown at this time. Identification is not quantitative.
CFU: Colony Forming Unit
PP: Polypropylene

Table A-2. Analytical Parameters and Target Limits

Matrix/Media:

Analytical Parameter	Project Action Limit/Level (applicable units)	Laboratory Limits[1] (applicable units)	
		Quantitation Limits	Detection Limits (if appropriate)
Fecal coliform	1 cfu/100 mL	1 cfu/100 mL	1 cfu/100 mL
Bacteriodes species/DNA	Detect	Detect	Detect

[1] Laboratory quantitation limits and detection limits are those that an individual laboratory or organization is able to achieve for a given analysis on a routine basis.

- Quantitation limits are the minimum concentrations that can be identified and quantified above the detection limit within some known limits of precision and accuracy/bias. It is recommended that the quantitation limit is supported by the analysis of a standard of equivalent concentration (typically, the lowest calibration standard).
- Detection limits are the minimum concentration that can be detected above background or baseline/signal noise of an instrument.

cfu: colony forming unit

Table A-3. Sampling Design and Rationale

Sampling Location/ID Number	Matrix/ Media	Depth (Appropriate Units)	Analytical Parameter1	Rationale for Sampling Design 2
614 North Oakland	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station not meeting NSSP standards for shellfish harvest
NE North Oakland	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station not meeting NSSP standards for shellfish harvest. Station at northern end of clam beds.
615 Chapman	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station threatened to not meeting NSSP standards for shellfish harvest. Determining if site is influenced by pollution from human sources.
639 Chapman	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station threatened to not meet NSSP shellfish harvesting standard.
UNC 3 Chapman	Fresh Water	Midpoint of stream depth at thalweg3	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
UNC 2 Chapman	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
UNC T Chapman	Fresh Water	Midpoint of stream depth at thalweg[3]	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
UNC 1 Chapman	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
CAM 1 Chapman	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
DEE O North Oakland	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
CRA 0 North Oakland	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
TR 03 North Oakland	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station

[1] Analytical parameters include all planned field measurements (e.g., dissolved oxygen, turbidity, pH, etc.), field screening analysis (e.g., PCBs by immunoassay test kit, selected metals by XRF), and laboratory analyses.

[2] Rationale supports the selection of sampling locations and associated analytical parameters.

3 Thalweg - the line of deepest water in a stream channel as seen from above, normally associated with the zone of greatest velocity in the stream channel.

Comment [SH3]: Can you draft the rationale for each of the sample sites and add to this??

Table A-4. Summary of Field and QC Samples To Be Collected

Matrix/ Media	Analytical Parameter[1]	No. of Sampling Locations	Depth[2] (surface, mid, or deep)	No. of Field Duplicates	Organic Analyses[3] No. of:		Inorganic Analyses[3] No. of:		No. of Transfer Blanks	No. of Equipment Blanks	No. of PE Samples[4]	Total No. of Samples
					MS	MSD	Dup	MS				
LABORATORY ANALYSES: See Section 2.4												
FIELD ANALYSES:												
FIELD MEASUREMENTS:												

[1] Analytical parameters include all laboratory analyses, field analyses (e.g., nutrients by various field test kits, PCBs by immunoassay test kit, select metals by XRF, etc.), and field measurements (e.g., dissolved oxygen, turbidity, pH, etc.).
[2] When samples are collected at different depths at the same location, information for each depth category (e.g., surface, mid, or deep/bottom) is provided on a separate line.
[3] Information includes the number of associated analytical QC samples, if collection of additional sample volume and/or bottles is necessary. If the QC samples listed are part of the analysis and don't require the collection of additional sample volume and/or bottles, ANAS@ (for Ano additional sample@) is included in the column. (Note: MS=matrix spike, MSD=matrix spike duplicate, Dup=laboratory duplicate/replicate.)
[4] PE or Performance test will be submitted for laboratory analysis along with the associated field sampled where noted.

Table A-5. Analytical Method, Containers, Preservation, and Holding Times Requirements

Matrix/Media: Ambient Water/Microbial

Analytical Parameter[1] and/or Field Measurements[2]	Analytical Method Number	Containers (number, size/volume, type)	Preservation Requirements (chemical, temperature, light protection)	Maximum Holding Times[3]
ANALYTICAL PARAMETER:				
Fecal coliform	Standard Methods 9221E(a)	250 ml	< 10 º C	24 hours
PCR	Appendix C	500 ml PP	< 10 º C	6 (2) hours
FIELD MEASUREMENTS:				

[1] Analytical parameter includes both field and laboratory analyses.
[2] Field measurement parameters include those parameters measured directly in the field (e.g., dissolved oxygen, turbidity, pH, etc.). Single 500 ml PP bottle provides adequate sample for all analyses conducted at the MEL.
[3] Maximum holding times include all pertinent holding times for each analytical parameter (e.g., from sample collection to sample preparation, from sample preparation to analysis, from sample collection to analysis, etc.) and field measurement (e.g., from sample collection to measurement).

Table A-6.2 Quality Control Requirements for Analyses

Analytical Method/SOP: PCR qualitative[2]

QC Sample:	Data Quality Indicator (DQI)	Frequency/ Number	Method/SOP QC Acceptance Limits	Acceptance Criteria/ Measurement Performance Criteria[1]	Corrective Action
LABORATORY ANALYSIS:					
Filtration control		1 per day	Negative, no DNA	Negative, No DNA	Data reviewed, decision made based on cause
Transfer blank		1 per day	Negative, no DNA	Negative, no DNA	Data reviewed, decision made based on cause
Positive control		1 per day	Positive reaction	Appropriate DNA Amplification	Data reviewed, decision made based on cause
Negative control		1 per day	Negative reaction	Appropriate DNA Amplification	Data reviewed, decision made based on cause
Replicate analysis		10 % of samples	Same result	Appropriate DNA amplification	Data reviewed, decision made based on cause
FIELD ANALYSIS:					
Not applicable					

2- See section 2.5.1 for additional QC requirements associated with the qualitative PCR analyses.

Table A-7. Quality Control Requirements for Field Measurements

Field Parameter:

QC Sample:	Data Quality Indicator (DQI)	Frequency/ Number	Method/SOP QC Acceptance Limits	Acceptance Criteria/ Measurement Performance Criteria[1]	Corrective Action
<<PARAMETER 1 – Instrument Name (Manufacturer, Model)>>					
Not applicable					
<<PARAMETER 2 – Instrument Name (Manufacturer, Model)>>					
Not applicable					
<<PARAMETER 3 – Instrument Name (Manufacturer, Model)>>					
Not applicable					

[1] Information supports the acceptance criteria/measurement performance criteria introduced in section 1.7.3.

Table A-8. Field Equipment/Instrument Calibration, Maintenance, Testing, and Inspection

Analytical Parameter	Field Equipment/ Instrument	Calibration Activity	Maintenance Activity	Testing/ Inspection Activity	Frequency	Acceptance Criteria	Corrective Action
Not applicable							

Appendix A-3. Data Quality Forms.

A-3.1. Attachment 1 – Sample Alteration Form

Project Name and Number: _____

Material to be Sampled:_____

Measurement Parameter:

_____

Standard Procedure for Field Collection & Laboratory Analysis (cite reference):

_____

Reason for Change in Field Procedure or Analysis Variation:

_____

Variation from Field or Analytical Procedure:

_____

Special Equipment, Materials or Personnel Required:

_____

Initiators Name: _____Date: _____

Project Officer: _____Date: _____

QA Officer: _____Date: _____

A-3.2. Attachment 2 – Corrective Action Form

Project Name and Number: _____

Sample Dates Involved: _____

Measurement Parameter:

_____

Acceptable Data Range:

_____

Problem Areas Requiring Corrective Action:

_____

Measures Required to Correct Problem:

_____

Means of Detecting Problems and Verifying Correction:

_____

Initiators Name: _____ Date: _____

Project Officer: _____ Date: _____

QA Officer:

APPENDIX B. FIELD DOCUMENTATION

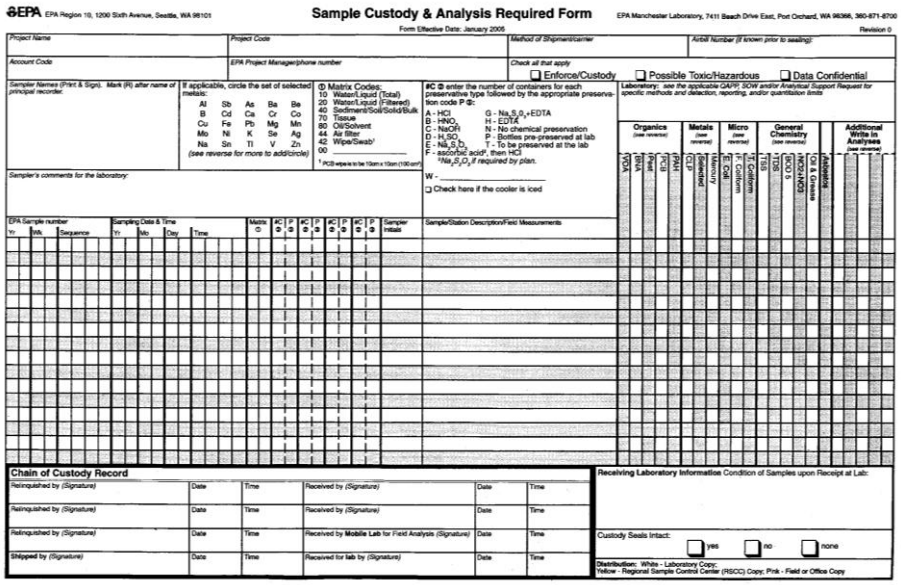
Appendix B-1. Equipment/Instrument Manual

Not applicable to this project.

Appendix B-2. Standard Operating Procedures

Not applicable to this project.

Appendix B-3. Field Data Forms and Chain-of-Custody Documentation



EPA Region 10, 1200 Sixth Avenue, Seattle, WA 98101

Sample Custody & Analysis Required Form

Form Effective Date: January 2005

EPA Manchester Laboratory, 7411 Beach Drive East, Port Orchard, WA 98366, 360-871-8700

Revision 0

Project Name | Project Code | Method of Shipment/carrier | Airbill Number (if known prior to sealing)

Account Code | EPA Project Manager/phone number | Check all that apply: ☐ Enforce/Custody ☐ Possible Toxic/Hazardous ☐ Data Confidential

Sampler Names (Print & Sign). Mark (R) after name of principal recorder.

If applicable, circle the set of selected metals:

Al	Sb	As	Ba	Be
B	Cd	Ca	Cr	Co
Cu	Fe	Pb	Mg	Mn
Mo	Ni	K	Se	Ag
Na	Sn	Tl	V	Zn

(see reverse for more to add/circle)

Ⓜ Matrix Codes:
10 Water/Liquid (Total)
20 Water/Liquid (Filtered)
40 Sediment/Soil/Solid/Bulk
70 Tissue
80 Oil/Solvent
44 Air filter
42 Wipe/Swab/
00 ____

* HCB-wipe is to be 10cm x 10cm (100cm²)

#C Ⓢ enter the number of containers for each preservative type followed by the appropriate preservation code P Ⓢ:

A - HCl
B - HNO_3
C - NaOH
D - H_2SO_4
E - $Na_2S_2O_3$
F - ascorbic acid*, then HCl
G - $Na_2S_2O_3$+EDTA
H - EDTA
N - No chemical preservation
P - Bottles pre-preserved at lab
T - To be preserved at the lab
*$Na_2S_2O_3$ if required by plan.

W - ____

☐ Check here if the cooler is iced

Laboratory: see the applicable QAPP, SOW and/or Analytical Support Request for specific methods and detection, reporting, and/or quantitation limits

Organics (see reverse): VOA, BNA, Pest, PCB, Oil
Metals (see reverse): CLP, Selected, Mercury
Micro (see reverse): E. Coli, F. Coliform
General Chemistry (see reverse): Tss, TDS, TOC, COD, NH3/CN/CR, Oil & Grease
Additional Write in Analyses (see reverse): Asbestos

Sampler's comments for the laboratory:

EPA Sample number Yr	Mth	Sequence	Sampling Date & Time Yr	Mo	Day	Time	Matrix Ⓜ	#C Ⓢ	P Ⓢ	#C Ⓢ	P Ⓢ	#C Ⓢ	P Ⓢ	#C Ⓢ	P Ⓢ	Sampler Initials	Sample/Station Description/Field Measurements

Chain of Custody Record

Relinquished by (Signature)	Date	Time	Received by (Signature)	Date	Time
Relinquished by (Signature)	Date	Time	Received by (Signature)	Date	Time
Relinquished by (Signature)	Date	Time	Received by Mobile Lab for Field Analysis (Signature)	Date	Time
Shipped by (Signature)	Date	Time	Received for lab by (Signature)	Date	Time

Receiving Laboratory Information Condition of Samples upon Receipt at Lab:

Custody Seals Intact: ☐ yes ☐ no ☐ none

Distribution: White - Laboratory Copy; Yellow - Regional Sample Control Center (RSCC) Copy; Pink - Field or Office Copy

Additional Matrix Codes: 30 Leachate 50 Sludge 60 Air

Matrix codes: these are the codes in use at the EPA Region 10 Laboratory. Pick the matrix code that best matches the sample matrix. If in the opinion of the sampler, the sample matrix needs to be specially described, select 00 and write in a matrix description. Remember, tissue can be animal or vegetable in nature.

If the write in area becomes filled, cross out one of the pre-printed analyses and write in what is needed. Try to use the bolded analyte symbol/abbreviation (some analyses are not abbreviated).

Organics pre-printed on the form:

PAH Polynuclear Aromatic Hydrocarbons (these are a subset of the compounds reported from GC-MS analyses for BNA - PAH by HPLC or SIM-GC/MS methods are usually requested in order to get low reporting limits). **Pest** Organochlorine Pesticides **PCB** Polychlorinated Biphenyls aka Aroclors **VOA** (aka VOC) - volatile organic compounds **BNA** (aka SVOC or SVOA) - semivolatile organic compounds

Organics that can be written in:

AED scan (detects chlorinated or brominated hydrocarbons) **Butyltins** Butyltins (mono, di, tri, tetra substituted) **CB Con** - Chlorinated Biphenyl Congener analysis **Chlor Hyd**. Chlorinated Hydrocarbons **Chlorophenols** **Gua/Cat** Guaiacols/Catechols scan **Herb** Herbicides **OP Pest** Organophosphorous Pesticides **PBDE** Polybrominated diphenylethers **Resin Acids** **TPH-Dx** Total Petroleum Hydrocarbons, diesel range **TPH-Dx-ext** Total Petroleum Hydrocarbons, diesel range extended to motor oil **TPH-Gx** Total Petroleum Hydrocarbons, gasoline range **TPH-HCID** Total Petroleum Hydrocarbons, identification **THMs** Trihalomethanes

Metals pre-printed on the form *(underlined = 'CLP metals' - mercury must be separately requested)*:

Al aluminum **Sb** antimony **As** arsenic **Ba** barium **Be** beryllium **B** boron **Cd** cadmium **Ca** calcium **Cr** chromium **Co** cobalt **Cu** copper **Fe** iron **Pb** lead **Mg** magnesium **Mn** manganese **Hg** mercury **Ni** nickel **K** potassium **Se** selenium **Ag** silver **Na** sodium **Sn** tin **Tl** thallium **V** vanadium **Zn** zinc

Metals that can be written in and then circled under the box used for designating selected metals:

Au gold **Cr+6** hexavalent chromium **Mo** molybdenum **Sr** strontium **Ti** titanium **W** tungsten **Zr** zirconium

Note: some metals may not be analyzed for on matrices other than soil/sed or water.

Microbiology Analyses pre-printed on the form:

E. Coli Escherichia coli **F. Coliform** Fecal Coliform **T. Coliform** Total Coliform

Microbiology Analyses that can be written in:

Enterococci **MPA** Microscopic Particulate Analysis for Determining GWUDI
G/C Giardia/Cryptosporidium **Coliphage** **Staph a** Staphylococcus aureus

Toxicity Characteristic Leaching Procedure (TCLP) write in analyses[3]:

TCLP BNA **TCLP Herb** TCLP Herbicides **TCLP met+Hg** TCLP metals including mercury **TCLP met** TCLP metals not including mercury **TCLP Hg** TCLP mercury **TCLP Pest** TCLP Pesticides **TCLP VOA**

[3] *Analyses are normally only conducted for analytes with a TCLP regulatory criteria.*

General analyses pre-printed on the form:

BOD Biochemical Oxygen Demand **NO_2+NO_3** Nitrite plus Nitrate **Oil & Grease** **TDS** Total Dissolved Solids **TSS** Total Suspended Solids

General analyses that can be written in:

Acidity **Alk** Alkalinity **TNH3** Ammonia **HCO_3** Bicarbonate **Br** Bromide **CO_3** Carbonate **COD** Chemical Oxygen Demand **Cl** Chloride **Color** Color **Cond** Conductivity **CN** Cyanide **CN-W&D** Cyanide, weak & dissociable **Flash** Flash Point **F** Fluoride **Grn Siz** Grain Size **Hard** Hardness **NO_2** Nitrite **NO_3** Nitrate **TNVS** Non-Volatile Solids **NVSS** Non-Volatile Suspended Solids **CLO_4** Perchlorate **pH** **Phenol** Phenolics **SiO_2** Silica - dissolved **SO_4** Sulfate **S** Sulfide **TOC** Total Organic Carbon **TS** Total Solids **% V Slds** % Volatile Solids **TVS** Volatile Solids **TVSS** Volatile Suspended Solids **SetSlds** Settleable Solids **% Tot** % Total Solids **TKN** Total Kjeldahl Nitrogen **T-Phos** Total Phosphorous **D-Phos** Dissolved Phosphorous **O-Phos** Ortho Phosphrous **D-O-Phos** Dissolved Ortho Phosphrous **Turb** Turbidity

Container guidance.

Note: this is general information only - consult the QA Project Plan on appropriate containers and preservatives for each project. Modifying methods may require modifying the number/type of containers. Freezing samples for one or more analyses may require collection of individual containers. Contact the laboratory for minimum sample volumes in situations where sample material is limited. Minimum volumes required for analysis will depend on the analysis and required reporting limits.

Containers for soil/sediment:

Metals/cyanide/mercury: 1, wide mouth 8 ounce glass or HDPE.
Extractable organics: 1, 8 ounce wide mouth amber glass, for one or two analyte groups
Inorganics and organics: 1, sixteen ounce wide mouth amber glass.
VOAs/purgeables: Contact the laboratory for the proper number/type of special Closed-System sample containers.

Containers/chemical preservatives for water[4]:

Metals/regular mercury: 1, one liter HDPE, HNO3 to pH<2
Mercury by method 1631: HCl and 250 mL containers provided by MEL
Cyanide: 1, 250 mL or larger HDPE, remove sulfides and/or residual chlorine then add NaOH to pH>12
Extractable organics (BNA, Pest, PCP, PAH etc.): two, one liter amber glass containers **for each analysis** - if more than one liter will be extracted for the project, it is advisable that the container size match (but not exceed) the volume to be extracted. Two separate volumes are usually collected for each analysis to allow for re-extraction if needed.
VOAs/purgeables: 3, zero headspace 40 mL amber glass vials with Teflon Septa, remove residual chlorine then add HCl to pH<2
Alkalinity: 1, 250 mL or larger HDPE, no extra volume for lab QC
Ammonia: 1, 250 mL or larger HDPE, H2SO4 to pH<2, no extra volume for lab QC
BOD 5: 1, one gallon HDPE, no extra volume for lab QC
TSS: 1, one liter or larger HDPE, no extra volume for lab QC
TDS: 1, 250 mL or larger HDPE, no extra volume for lab QC
Oil & Grease: 1, one liter clear glass, HCl to pH<2, submit 4 **separate** containers for the lab QC sample
NO2+NO3: 1, 250 mL or larger HDPE, H2SO4 to pH<2, no extra volume for lab QC
Br, Cl, F, SO4, CLO4: for analysis by ion chromatography, 1, 100 mL or larger HDPE, no extra volume for lab QC

[4] *Water samples to be designated for lab QC should have double volume submitted for metals, triple volume for organics. In general, extra volume is usually not required for lab QC for soil/sediment.*

APPENDIX C. LABORATORY DOCUMENTATION

Appendix C-1. QA Manual

Laboratory analysis and procedures will comply with the guidelines described in the document entitled, *Quality Assurance Manual for the U.S. EPA Region 10 Manchester Environmental Laboratory (October 2005).* The QA Manual is available at the following website on EPA's Intranet (G:\Sops\NELAC 2005 QAM\NELACTable.htmL). If you are unable to access this document and would like to obtain a copy, please contact Stephanie Harris.

Appendix C-2. Standard Operating Procedures.

C-2.1. SOP for recreational and waste water DNA purification

NRMRL-CI-SOP- _____

DATE _____January 25, 2006_____

REVISION # __0___DATE_____

BRANCH/DIV_____MCCB/WSWRD___

Standard Operating Procedure (SOP):

Recreational Water and Wastewater Filtration for DNA Extractions

SCOPE AND APPLICATION

This procedure instructs user in how to filter recreational and wastewater samples for microbial DNA extraction.

APPROVALS

Originator/User:	Orin C. Shanks
Branch Chief:	Mark Rodgers
Division Director:	Jim Goodrich
Quality Assurance Officer:	Steve Harmon

Equipment and Supplies:

1. Sterilized gloves.
2. Disposable Filter unit: Analytical Test Filter Funnel (Catalog #: 09-740-30G; Fisher Scientific).
3. Vacuum manifold that can accommodate a 47 mm diameter filter.
4. #8 silicone stopper with 0.365 inch hole (Catalog #: XX2004718; www.millipore.com)
5. Vacuum pump or other vacuum source.
6. Filter forceps.
7. Sterilization source for forceps (10% bleach with Ultrapure water rinse or immerse in 95% ethanol and flame).
8. Ultrapure water (Catalog #: TMS-006-A; Specialty Media, Phillipsburg, NJ).
9. Supor-200 membrane filters, 0.2µm pore size, 47mm diameter (PALL, Ann Harbor, MI).
10. Polycarbonate filters, 0.4 µm pore size, 47mm diameter (Catalog #: K04CP04700; Osmonics Inc.)
11. GITC buffer [5M Guanidine isothiocyanate, 100 mM EDTA (pH8), 0.5% Sarkosyl].

Recreational Water and Wastewater Handling:

Recreational water or wastewater samples should be chilled immediately following collection, and stored at a temperature ranging from 0-10°C until time of filtration.

Duplicates and Controls:

1. Two aliquots from each sample will be filtered and assayed individually (sample duplicate).
2. Two filtration negative controls for batch of samples (batch = 1-20 samples).

Disposal:

After filtration, recreational water and wastewater can be discarded in the sink.

Safety Measures:

1. Wear gloves. Always treat unfiltered samples as if they are contaminated with pathogens. Keep them contained and clean up with bleach solution.

2. GITC buffer is toxic; avoid contact with skin. Standard good lab practice should always be followed. Consult the Material Safety Data Sheet for information on hazards

associated with undiluted powdered GITC. Most safety precautions for guanidine isothiocyanate apply to undiluted powdered GITC; workers mixing the stock solutions should use a mask and skin protection and work under a hood with spill protection.

Store 5M GITC at room temperature in the dark. Aqueous 5 M GITC buffer has negligible vapor pressure and so does not present a hazard from fumes, and presents negligible hazard from splashing. Workers should wear nitrile gloves and skin protection. If there is a spill, it should be mopped up with paper towels that should then be placed in a plastic bag. Broken or leaking tubes should also be bagged and disposed of according to laboratory regulations. Wipe the work area with a wet paper towel after a spill to minimize dust.

Protocol:

1. Remove filter funnel from bag. Attach funnel adapter by pressing firmly in place and securely seat assembled unit in stopper. Then insert entire filter apparatus into a manifold or vacuum flask. To remove the stock filter membrane, firmly grip blue collar with thumb and forefinger. Grip funnel top with other hand. With fingers, gently squeeze funnel top into an oval to break seal. Peel funnel top away in one motion to expose the stock membrane. DO NOT TWIST! Aseptically remove the stock membrane. For recreational water samples, aseptically install a 0.2 µm pore size 47mm diameter Supor-200 membrane (shiny side down). For wastewater samples, aseptically install a 0.4 µm pore size 47mm diameter polycarbonate membrane. Be sure to leave behind the filter pad underneath the membrane. Reassemble filtration apparatus. Connect vacuum source to designated area on vacuum manifold or vacuum flask.

2. Pour 25 ml of mixed wastewater sample, 100 ml of recreational water, or 100 ml filtration control into the filtration unit and apply a vacuum until water has completely passed through the filter. Rinse the sides of the filtration apparatus with 10 ml of Ultrapure water to capture any remaining residues. Note: Perform filtration controls using the same filter type used for wastewater or recreational samples (see Step 1.).

3. Turn off the vacuum and dismantle the filtration unit.

4. Carefully roll up the contaminated filter using sterile filter forceps and/or a sterile toothpick. Do not touch the contaminated surface to the base of the filtration unit.

5. Place the rolled filter into a 15 ml tube containing 0.5 ml GITC buffer. Ensure that the filter goes to the bottom.

6. Gently invert the tube several times to ensure that the entire filter is wet with buffer.

7. Label the tube and place the tube into a freezer (-20°C) for storage. Label information should include origin of sample (ie. name of wastewater facility), date, and technician initials.

8. Sterilize the forceps using 10% bleach with Ultrapure water rinse or immersion in 95% ethanol and flame. To filter a duplicate sample, place a fresh filter into the filtration unit using sterilized forceps and reassemble Analytical Test Filter Funnel unit. Please note that the same filter type should be used for duplicate samples (see step 1.).

9. Repeat process (steps 2-8) with duplicate sample. After all duplicate samples are filtered, sterilize forceps (10% bleach with Ultrapure water rinse or immerse in 95% ethanol and flame) and discard filtration unit.

10. For each sampling batch (1-20 samples processed on the same day), prepare 2 filtration negative controls (single filter unit with duplicate filters) by following the above procedure, but substituting 100 ml Ultrapure water for a recreational water or wastewater sample.

C-2.2. Scat Fecal Sample DNA Extraction

Fecal DNA Extraction

Equipment:
-Pipetmen pipettors (p200 and p1000) with barrier tips
-Vortex mixer
-Bead Beater Instrument
-Heating block or hybridization oven (60oC)
-Microcentrifuge (14,000 x g)
-2.0ml and 0.65ml low-adhesion microtubes
-1ml disposable transfer pipets
-15ml Falcon tubes

Reagents:
-FastDNA Kit (Q-BIOgene # 6540-400)
-GITC Buffer (5M Guanidine isothiocynate, 100mM EDTA pH 8.0, 0.5% Sarkosyl)
-AE Buffer (Qiagen)

Protocol:

1. Place 2g feces (wet weight) into 15ml falcon tube.
2. Add 5ml GITC buffer, vortex, and incubate at room temperature for five minutes [up to 5 hours is acceptable].
3. Add 800ul CLS-VF and 200ul PPS into a bead beating microtube.
4. Add 200ul of GITC buffer/fecal slurry into bead beating microtube [use disposable transfer pipet].
5. Agitate with bead beater for 30 seconds and centrifuge for 5 minutes at 14,000 g.
6. Pipet 600ul of extract supernatent from bead beating microtube and 600ul binding matrix into 2.0ml low-adhesion microtube, incubate at room temperature for 5 minutes [agitate gently], and centrifuge for 1 minute at 14,000 x g.
7. Discard supernatent and wash pellet with 500ul SEWS-M [make sure to add ethanol], resuspend pellet, and centrifuge for 1 minute at 14,000 x g.
8. Repeat step 7.
9. Dry silica pellet with 60oC incubation for 5 minutes.
10. Elute DNA with 100ul AE buffer [Qiagen Dneasy Kit], mix gently, incubate at 60oC for 5 minutes, centrifuge for 5 minutes at 14,000 x g, remove supernatent, and store in 0.65ml low-adhesion microtube at -20oC.

C-2.3. Laboratory Reagent Methods

Lab Reagents/Recipes

Orange G
15.0 g sucrose
0.175 g Orange G -- Sigma 3756
MilliQ water to 50 mL

25% Acteamide
2.5 g acetamide
10 mL MilliQ water

Primer Dilutions
(dilute to be 25 pmol/µl since that is the amount we use in a PCR reaction)

Note concentration of primer (from primer vial; usually nmol concentration)

nmol = 10^{-9}
pmol = 10^{-12}

conc.of primer in nmol X 1000 pmol/nmol ÷ 25 pmol/µl

will give you the amount of distilled water to add to the primer to give a final concentration of 25 pmol/µl

LB (Luria-Bertani) Medium and Plates
In a 1L bottle dissolve 7.75g LB Broth Media in 500 mL deionized water
Autoclave on liquid cycle for 15 minutes at 15 psi.
Allow solution to cool to 55ºC.
Add 50mg ampicillin (add the ampicillin to ~5ml of water and use a 0.22µm syringe filter to add to media). Mix.

LB Agar Plates
LB medium as above, but add 7.5g agar before autoclaving (boiling is recommended to help agar go into solution). After autoclaving, cool to ~55ºC, add ampicillin and pour into petri plates. Let harden, invert and store at 4ºC, in the dark.

C-2.4. Off-Site Collaborator Fecal Sample Collection Protocol

Off-Site Collaborator Fecal Sample Collection Protocol

Project Overview:
The goal of my project is to study the fecal microorganism communities from different animals. Once the fecal material passes out of the animal, the microbial community starts to change in response to its new environment. Therefore, it is important to collect fresh fecal samples when possible. It is also important to make sure that each fecal sample represents one individual.

Fecal Sampling Protocol:
Fecal sample amounts should range from approximately 5-10 grams (wet weight). Do not spend time massing samples just take your best guess. Wear fresh sterilized latex gloves for each individual sample collected. Place the fecal material into a sterilized 50 ml conical tube using a disposable spatula, label the tube (animal species, date, and estimate time on the ground), store on ice in the field, and freeze at -20°C as soon as possible. Use a fresh spatula for each fecal sample. Some fecal samples will be too large to fit in the 50 ml conical tube. For larger samples, collect a manageable amount, seal in a ziplock bag, and label as described above. Once in the laboratory, collect a small portion of the fecal material and place in 50 ml conical tube using a sterilized disposable spatula. Otherwise, you can mail the large samples in the ziplock bags. What ever is the easiest will work for me. Please keep in mind that the samples will need to be shipped in dry ice and that small tubes are easier to handle and ship.

Equipment Supplied by MCCB:
I will arrange to have a minimum of 100 50 ml conical tubes, a small cooler, and disposable spatulas sent to your lab. You will need to provide sterile gloves and ziplock bags.

Shipment of Samples:
Samples should be shipped in an insulated cooler (no Styrofoam). Each sample must be stored in a leak-proof primary container (50 ml conical tube) and sealed with tape or Parafilm®. Each sealed primary container must be individually wrapped in an absorbent material such as a paper towel. This step is required for shipment of diagnostic samples and helps to prevent mixing of the samples in case of leaks. Primary containers should be stored inside a secondary container (ziplock bag). Please pack samples with several frozen ice packs and overnight FedEx immediately. The FedEx package does not require a special label or other markings noting the contents of the package. The FedEx account number is 293966389. Do not use the FedEx number for any other purposes (I have to say this). Please ship samples on a Monday or Tuesday and mail the samples to:

Orin Shanks
US EPA
26 West Martin Luther King Drive
MS 387
Cincinnati, OH 45268

Orin C. Shanks
U.S. Environmental Protection Agency
Last Updated: January 23, 2006

C-2.5. PCR and Gel Electrophoresis Methods

TABLE 1. Primers

Primer	Sequence (5'-3')	Target
Bac32F	AACGCTAGCTACAGGCTT	*Bacteroides-Prevotella*
Bac708R	CAATCGGAGTTCTTCGTG	*Bacteroides-Prevotella*
CF128F	CCAACYTTCCCGWTACTC	ruminants
CF193F	TATGAAAGCTCCGGCC	ruminants
HF134F	GCCGTCTACTCTTGGCC	human
HF183F	ATCATGAGTTCACATGTCCG	human

HF, human-specific; CF, ruminant-specific. Numbers correspond to the numbers of the *E. coli* 16S rRNA gene. All forward primers are paired with Bac708R.

***Bacteroides* PCR**

Cycler Conditions for General *Bacteroides* Assay

Step 1. Incubate at 94°C for 2 minutes
Step 2. Incubate at 94°C for 1 minute
Step 3. Incubate at 53°C for 1 minute
Step 4. Incubate at 72°C for 1 minute
Step 5. Go to step 2 (34 more times)
Step 6. Incubate at 72°C for 5 minutes
Step 7. Incubate at 4°C forever

Cycler Conditions for Ruminant *Bacteroides* Assay

Step 1. Incubate at 94°C for 2 minutes
Step 2. Incubate at 94°C for 1 minute
Step 3. Incubate at 62°C for 1 minute
Step 4. Incubate at 72°C for 1 minute
Step 5. Go to step 2 (34 more times)
Step 6. Incubate at 72°C for 5 minutes
Step 7. Incubate at 4°C forever

Alpha Innotech Alpha quant 6
Cat 60-13069-00
www.Alphainnotech.com

orange G.

Cycler Conditions for Human *Bacteroides* Assay

Step 1. Incubate at 94°C for 2 minutes
Step 2. Incubate at 94°C for 1 minute
Step 3. Incubate at 63°C for 1 minute
Step 4. Incubate at 72°C for 1 minute
Step 5. Go to step 2 (34 more times)
Step 6. Incubate at 72°C for 5 minutes
Step 7. Incubate at 4°C forever

4µL/200mL agarose

Agarose Gel

Prepare a 1% agarose gel using 0.5X of TBE. Agarose can be dissolved by heating the solution on a hot plate or a microwave. Do not allow the solution to spill over, otherwise you are changing the concentration of agarose. Cool agarose to 50-55°C in an incubator and add adequate amount of GelStar (3-4 µl per 100ml of agarose; www.cambrex.com/). This is our DNA stain of choice as it is more sensitive than conventional stains like ethidium bromide. Since GelStar contains a nuclei acid stain, treat it as potentially carcinogenic, thus wear gloves while handling the gels. We run ours gels for one hour at 75-100 V. The running time will depend on the percentage of agarose, buffer, and type of gel box used. The expected size of the PCR products is approximately 700 bp for the general *Bacteroides* assay and between 515 and 580 bp for the host specific assays.

References

Bernhard AE and Katharine G. Field. 2000. A PCR Assay To Discriminate Human and Ruminant Feces on the Basis of Host Differences in *Bacteroides-Prevotella* Genes Encoding 16S rRNA. Appl. Envir. Microbiol. 66: 4571-4574.

Simpson, J. M., J. W. Santo Domingo, and D. J. Reasoner. 2002. Microbial Source Tracking – State of Science. Environ. Sci. & Tech. 36:5279-5288.

Appendix C-3. Data Report Forms.

C-3.1. Bacteriodes PCR Analysis Report Form

PCR Setup Sheet _____ Cycle Method: _____ - _____ - _____ - _____ - _____

Exp. # _____

Date: _____

	Time	Temp
Hold:	_____	_____
Denat:	_____	_____
Anneal:	_____	_____
Extend:	_____	_____
Extend Hold:	_____	_____
Final Extend:	_____	_____

No. Cycles _____

Reagents	Name	Lot. No.	ul	1	2	3	4	5	6	7	8	9	10
Dist. Water		_____	_____										
10X Buffer II		_____	_____										
dNTP mix		_____	_____										
25 mm MgCI		_____	_____										
Primer 1	_____	_____	_____										
Primer 2	_____	_____	_____										
Template	_____	_____	_____										
DNA Pol.		_____	_____										
Total volume			_____										

				11	12	13	14	15	16	17	18	19	20
Dist. Water		_____	_____										
10X Buffer II		_____	_____										
dNTP mix		_____	_____										
25 mm MgCI		_____	_____										
Primer 1	_____	_____	_____										
Primer 2	_____	_____	_____										
Template	_____	_____	_____										
DNA Pol.			_____										
Total volume			_____										

				21	22	23	24	25	26	27	28	29	30
Dist. Water		_____	_____										
10X Buffer II		_____	_____										
dNTP mix		_____	_____										
25 mm MgCI		_____	_____										
Primer 1	_____	_____	_____										
Primer 2	_____	_____	_____										
Template	_____	_____	_____										
DNA Pol.			_____										
Total volume			_____										

Data to Input

_____ Collection Site
_____ Date/Time Collected
_____ Species
_____ Approx. Age of Sample
_____ Sample # Assigned

_____ Date Purified
_____ Date Analyzed/PCR
_____ PCR Reaction #
_____ Confirmation Status

APPENDIX D. DATA EVALUATION

Appendix D-1. Data Evaluation/Documentation Form.

D-1.1. Microbiology Laboratory Data Review/Release Form

Project: Project Code:_____
Sample Numbers
Peer Reviewed by: Date:_____

Raw Data/Quality Control Check

____ Verify positive and negative culture controls associated with media are satisfactory.
____ Verify media sterility was checked.
____ Check for sample carryover/contamination if membrane filtration method used. Note any deficiencies.
____ Check duplicate analyst counts are within 20 %, when applicable.
____ Verify that media was prepared within method specifications.
____ Verify that samples were received and analyzed within the holding time.

Bench Sheet Check

____ Is the data package properly labeled?
- __ Analyst name
- __ Sample numbers and project name
- __ Analytical method used
- __ Date and time of collection/analysis

____ Verify that there is a bench sheet for each sample listed on the Analysis Required forms.
____ Verify that there is a Data Review Memo written for the project -forwarded to ESAT Data Entry Technician
____ Verify that there is a Data Release Memo for this project - forwarded to ESAT Data Entry Technician

Results

____ Verify that the reported results:
- __ have appropriate qualifiers assigned
- __ reflect the correct units
- __ reflect dilution factors used in the analysis
- __ were transferred correctly from the bench sheets
- __ were calculated correctly