UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 10 LABORATORY
7411 Beach Dr. East
PortOrchard, Washington 98366

MEMORANDUM

DATE: August 24, 2011

TO: Jo Henry, Project Officer
Washington Operations Office, USEPA Region 10

FROM: Stephanie Harris, DVM, DACVPM
Technical Director, Microbiology
Office of Environmental Assessment, USEPA Region 10 Laboratory

SUBJECT: Preliminary report for the SamishWatershed Microbial Source Tracking (MST) Project
Project Code: WOO-069A
Account Code: 0910B10P202BD4C24

cc: Gerald Dodo, Supervisory Chemist
Office of Environmental Assessment, USEPA Region 10 Laboratory

Sally Lawrence (slaw461@ecy.wa.gov)
Rick Haley (rickh@co.skagit.wa.us)

The following is a preliminary report discussing the results of the *Bacteroides* Polymerase Chain Reaction (PCR) analysis of selected samples from eleven (11) sampling events associated with the Samish Watershed MST project. The analyses were performed following the Standard Operating Procedures (SOP) identified in the associated Quality Assurance Project Plan (QAPP) developed by Washington Operations Office (WOO) with the assistance of the USEPA Region 10 Laboratory. This report is relevant for the following samples:

Sample numbers: 10114050 - 4067, 101444250-4252, 10154100 – 4116, 10194150 -4166, 10194168 – 4169, 10224200 – 4221, 10294400 - 4404, 10294406 – 4410, 10294412 – 4418, 10314300 – 4304, 10314306 – 4310, 10314312, 10314314- 4318, 10354350 – 4354, 10354356 – 4357, 10354359 – 4367, 10394400 – 4418, 10434450 – 4463, 10434465 – 4467, 11015001 – 5019, inclusive.

1.0 Sample Analysis and Determination of Results:
The Region 10 Laboratory conducted the following steps in analyzing the samples collected by Skagit County personnel.
1.1 Sample filtration within 24 hours of sample collection
1.2 Filter placed in sterile tube; preservative added and frozen at – 20 ºC
1.3 DNA extraction/purification performed using FastDNA® kit
1.4 Each sample tested for presence of appropriate DNA target using master mix and primer sets which are specific to DNA segments associated with *Bacteroides* (general), human *Bacteroides* and

ruminant *Bacteroides.* There are five primer sets utilized for this project: 1 general, 2 human sets and 2 ruminant sets of target DNA sequences.
1.5 Visualization of amplified DNA product using gel electrophoresis and UV trans-illumination.
A sample was considered negative for the presence of *Bacteroides* if all five concentrations of the DNA extract from the sample (as processed through steps 1.4 – 1.5) provided negative results. If at least one of the five concentrations of the DNA extract produced a positive result with one or both of the *Bacteroides* human primer sets, the sample was considered to be positive for human fecal contamination. If at least one of the five concentrations of the DNA extract produced a positive result with one or both of the *Bacteroides* ruminant primer sets, the sample was considered to be positive for ruminant fecal contamination.

2.0 Quality Control Tests Performed:

As established in the Quality Assurance Project Plan for this project, the following quality control tests were conducted as an integral part of these analyses:

2.1 Positive DNA Controls (consisting of plasmid DNA containing the target sequence): A positive control was analyzed in conjunction with each set of amplifications and always provided an appropriate response for the data which is provided in this report.

2.2 Replicate Analyses: Filter replicates and field replicates (approximately 10 % of total samples) were analyzed for this project. Not all duplicates provided the identical results.

2.3 Blind Samples: Blind samples were provided and analyzed with this portion of the project. Results are provided in the data.

2.4 Negative Controls:

2.4.1 Extraction Negative Controls: Each time a batch of samples was extracted (step 1.3), a negative extraction control (DNA-free water used instead of sample) was extracted at the same time. These negative controls always provided an appropriate response.

2.4.2 PCR Negative Control (consisting of master mix and the appropriate primer set, but using water instead of sample): A negative PCR control was analyzed with each set of amplifications and always provided an appropriate response for the data which is provided in this report.

2.4.3 Filtration Controls: This control consisted of preparing an in-house filtration control and analyzing the resulting filter (steps 1.1-1.5). The filtration controls analyzed were negative for each of the sample sets completed for this report.

3.0 General Conclusions and Disclaimers:

3.1 All samples were tested for the presence of *Bacteroides*-specific DNA.

3.2 The species-specific primer sets that were used in this project were restricted to the identification of human and ruminant sources. The presence of general *Bacteroides* in a sample, combined with absence of both human and ruminant target DNA in that sample indicates that the fecal contamination present was neither human nor ruminant, but is associated with the presence of fecal contamination from another species of animal.

3.3 The presence of general *Bacteroides* target DNA in a sample and the presence of the human target DNA indicate that the fecal contamination was human. The presence of general *Bacteroides* target DNA in a sample and the presence of ruminant target DNA indicate that the fecal contamination was ruminant. The presence of general *Bacteroides* target DNA and absence of human and ruminant target DNA indicates that the fecal contamination was due to animals other than those tested. The presence of general *Bacteroides* target DNA in a sample and the presence of both the

human and ruminant target DNA indicate that the fecal contamination was human and ruminant. To be noted, there may be fecal contamination from other species present in these samples as well.

3.4 Target DNA segments that are extracted from fecal material from ruminants will provide a positive reaction to ruminant primer sets. Ruminant as defined here include cattle, sheep, deer, elk, and other animals with similar digestive tracts.

3.5 Analyses of the 169 samples collected (doesn't include blind samples or duplicates) demonstrated 23 samples positive for human-only biomarker (13.6 %), 24 samples positive for ruminant only biomarker (14.2 %) and 11 samples positive for both human and ruminant fecal source (6.5 %). There were 11 samples that tested negative for the *Bacteroides* screening test (6.5%) and 100 (59.2%) samples with general *Bacteroides*-only identified. This latter category indicates that human and ruminant source were not identified but that some other source is present in those samples and could not be identified. A number of sites demonstrated repeat hits for either human or ruminant or both sources. These are provided in table format at the end of this report. In addition, a cluster of ruminant source identification occurred on the June 2010 sampling at sites designated as Samish Bay cnnl, ED1 and SAM1, which appear to have been collected consecutively. Sites that have repeat positive results warrant additional investigation and might benefit from a concurrent shore side or land based inspections associated with future sampling events. Seasonality of human source demonstrated more prevalence in June and August through October sampling events, with an additional spike in January. Ruminant source was identified more frequently in the summer months (June – August), with another spike in January. Some sites demonstrated the noteworthy absence of either human or ruminant source, but the presence of general (or screening) *Bacteroidales* source. These sites include 03-FRI-008, 03-MCE-gate, 03-PAR-00.0, 03-SAM-10.3, 03-THO-003, and S Edison Drainage. The addition of more host specific markers may help to identify the animal fecal source(s) providing this contribution.

3.6 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 area. 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 our investigation into the specificity and sensitivity of these primers with known ruminant and non-ruminant species is on-going, to date our false positive rate is 3.6 % and false negative rate is zero. An example of a false positive would be a horse scat sample testing as positive for human source; an example of a false negative result would be a cow that tested negative for ruminant source.

Abbreviations used in data report:

3.7.1 ND - Analysis Not Done
- 3.7.1.1 Used when one of the primers in a set of two was positive. E.g. If HF 183 was positive for a sample, then analysis for HF 134 was not generally done.
- 3.7.1.2 Used when a sample tests negative for GB and no further testing is done.

3.7.2 R– Identifies the contaminant as coming from a ruminant source.

3.7.3 H– Identifies the contaminant as coming from a human source.

3.7.4 H/R- Identifies the contaminant as coming from both human and ruminant sources.

3.7.5 DU - Sample number followed by DU - indicates that the sample was analyzed in duplicate.

3.7.6 GB – identifies the contaminant as coming from an unknown fecal source (other than human or ruminant).
3.7.7 A – Absent (no *Bacteroides* biomarker, as indicated, was detected)
3.7.8 P – Present (identification of *Bacteroides* spp as indicated).

Sites with Repeated Source Identification

Site description	Ruminant repeat (y/n)	Human repeat (y/n)
0-3 SAM-16.5	N	Y
03-SED pump	Y	N
03-Thomas-03.6	Y	N
Colony creek	Y	N
Friday Creek	N	Y
McElroy slough ditch	N	Y
Parsons Creek	Y	Y
Samish R above Parsons	N	Y
Samish R at Thomas Rd	Y	Y
Thomas Cr at F&S	N	Y
Thomas Cr @ 99	N	Y
Weir Cr	Y	Y
Samish R at Grip Rd	Y	N
Samish R at 99	N	Y