

APPENDIX A

Quality Assurance Report

Quality Assurance Report

The overall quality assurance objective for measurement data was to ensure that data of known and acceptable quality were obtained. All sampling, isolation, and analytical testing were to be performed to yield consistent results that are representative of the media and conditions measured. All data were to be recorded and reported in terminology consistent with that of other agencies and organizations to ensure comparable results. A complete description of the quality assurance procedures can be found in the sampling and analysis plan (Herrera 2003). The following sections include a description of the fecal coliform bacteria and ribotyping data quality assessment procedures and results.

Bacterial Enumeration

No major quality control problems were identified in this study. Values associated with minor quality control problems were considered estimates and assigned J or G qualifiers (see explanation below). Estimated values were used for evaluation purposes. The following section describes data assessment procedures, quality control problems, and corrective actions taken for the following quality control elements:

- Completeness
- Methodology
- Holding times
- Blanks
- Laboratory duplicates
- Field duplicates.

Completeness

A total of 80 water samples were collected in accordance with the sampling and analysis plan. All water samples were analyzed for fecal coliform bacteria and resulted in valid data. No recollection or reanalysis of samples was required.

Methodology

Methodology was assessed by examining the field notebook and laboratory reports. No substantial deviations from the procedures described in the sampling and analysis plan (Herrera 2003) were noted based on this review, and no data were rejected as a result.

The membrane filtration method used to measure bacterial concentrations requires that between 20 and 60 colonies are present on the culture plate to achieve the most accurate count. In this study, 39 out of 80 water sample bacteria results (49 percent) were reported as estimates because

fewer than 20 colonies were present on the culture plate used for enumeration. These results were assigned a *J* qualifier by the quality assurance officer (see database in Appendix C). Five of the 80 water samples (6 percent) were reported as “greater than” the measured value because greater than 60 colonies were present on the culture plate. These results were assigned a *G* qualifier by the quality assurance officer (see Appendix C).

Holding Times

All water samples were delivered to the laboratory and analyzed on the day of collection, which meets the maximum holding time (24 hours) established by the sampling and analysis plan for bacteria analysis.

Blanks

Three laboratory blanks were analyzed with each sample batch. One blank was filtered before the first water sample, one blank was filtered after the tenth water sample, and one sample was filtered after the last water sample. All 60 blank samples exhibited a concentration of less than the detection limit (2 colony forming units/100 mL).

Laboratory Duplicates

Laboratory duplicates were analyzed for one water sample per sample date (batch) to measure the precision of the analysis. Precision of the laboratory duplicate results were calculated according to the following equation:

$$RPD = \frac{(C_1 - C_2) \times 100\%}{(C_1 + C_2) / 2}$$

where:

- RPD = relative percent difference
- C₁ = larger of two values
- C₂ = smaller of two values.

Two levels of precision for duplicate analyses were evaluated. The relative percent difference (RPD) of laboratory duplicates shall be less than or equal to 25 percent for values greater than five times the detection limit (i.e., greater than 10 CFU/100 mL). If either duplicate value is less than or equal to five times the detection limit (i.e., less or equal to than 10 CFU/100 mL) then the difference between duplicates shall be within two times the detection limit (i.e., shall not exceed 4 CFU/100 mL).

Table A1 presents the results of laboratory duplicate analyses. Of the 20 duplicates analyzed, eight duplicates did not meet the analytical precision objectives. However, sample results for four of those eight duplicates had been qualified as estimates (*J*) because less than 20 colonies

were counted. Sample results for the remaining four duplicates were qualified as estimates (J) because the precision criteria were not met.

Table A1. Laboratory duplicate data for fecal coliform bacteria analyses conducted for the Upper Willapa River Microbial Source Tracking Study.

Sample ID	Sample Result (CFU/100 mL)	Duplicate Result (CFU/100 mL)	Relative Percent Difference	Absolute Difference (CFU/100 mL)	Action
WRLE 111803-1	1020	1240	19	NA	None; objective met
WRCL 120203-2	2 U	2 U	NA	0	None; objective met
WRPA 121503-1	2 U	2 U	NA	0	None; objective met
WRCL 012104-1	14 J	6 J	NA	8	None; sample result qualified (J)
WRCL 012804-1	20 J	40 J	67	NA	None; sample result qualified (J)
WRCL 020904-1	6 J	8 J	NA	2	None; objective met
WRCL 022504-1	26 J	30 J	14	NA	None; objective met
WRCL 031504-1	5 J	7 J	NA	2	None; objective met
WRCL 032404-1	198	150	28	NA	Qualify sample results J
WRCL 041404-1	48	60	22	NA	None; objective met
WRCL 051704-1	32 J	40	22	NA	None; objective met
WRCL 052604-1	116	122	5	NA	None; objective met
WRCL 060704-1	72	54	29	NA	Qualify sample results J
WRCL 071204-2	20 J	40	67	NA	None; sample result qualified (J)
WRCL 081104-1	120 J	120	0	NA	None; objective met
WRCL 082504-1	1100	1140	4	NA	None; objective met
WRCL 090104-1	200	40 J	133	NA	Qualify sample results J
WRCL 101904-1	340	380	11	NA	None; objective met
WRCL 102504-1	40	2 U	NA	38	Qualify sample results J
WRCL 110104-1	100 J	40 J	86	NA	None; sample result qualified (J)

Bold indicates values that exceed quality assurance objective: relative percent difference ≤ 25 percent or absolute difference ≤ 4 CFU/100 mL

NA = Not applicable

U = Value less than detection limit shown

J = Estimated value due to less than 20 colonies counted.

Field Duplicates

To evaluate precision of sample collection and analysis, field duplicates were analyzed on two occasions. Duplicates were collected at the downstream station (WRC1) on February 25, 2004 and at the midstream station (WRLE) on August 11, 2004. The relative percent difference (RPD) values were 21 and 11 percent, respectively. Both RPD values meet the quality assurance objective for laboratory duplicates (i.e., less than or equal to 25 percent). (The collection of field duplicates was not specified in the sampling and analysis plan).

To assess environmental variability, consecutive grab samples were collected at the downstream station (WRC1). During each of the twenty sampling events, consecutive grab samples were

collected at least 10 minutes apart. Table A2 presents the results of the consecutive grab sample analyses as well as the calculated relative percent difference. Sample pairs are presented by base flow and storm flow.

Table A2. Consecutive grab data at the upstream station (WRC1) for fecal coliform bacteria analyses conducted for the Upper Willapa River Microbial Source Tracking Study.

Sample Date	Fecal Coliform Bacteria (CFU/100 mL)		Relative Percent Difference	Absolute Difference (CFU/1000 mL)
	Grab 1	Grab 2		
Base Flow				
12/2/03	64	24 J	91	NA
12/15/03	20	20 J	0	NA
1/21/04	14 J	15 J	14	NA
2/9/04	6 J	5 J	NA	1
2/25/04	26 J	20 J	26	NA
3/15/04	5 J	9 J	NA	4
5/17/04	32 J	20 J	46	NA
6/7/04	72 J	66	9	NA
7/12/04	38 J	20 J	62	NA
8/11/01	104	62	51	NA
Storm Flow				
11/18/03	960	580	49	NA
1/28/04	68	40 J	52	NA
3/24/04	240 J	300 J	22	NA
4/14/04	48	70	37	NA
5/26/04	116	86	30	NA
8/25/04	1100	1100	0	NA
9/1/04	124 G	124 J	0	NA
10/19/04	340 J	260 J	27	NA
10/25/04	54	64	17	NA
11/1/04	46	54	16	NA

Bold indicates values exceed quality assurance objective for laboratory duplicates: relative percent difference ≤ 25 percent or absolute difference ≤ 4 CFU/100 mL

NA = Not Applicable

G = Estimated value greater than value shown

J = Estimated value due to less than 20 colonies counted

Quality assurance objectives for laboratory duplicates were not met for 50 percent of the consecutive grab samples (i.e., five of the base flow samples and five of the storm flow samples). These data suggest that environmental variability of fecal coliform bacteria concentrations are similar for base and storm flow samples, and are similar to the analytical variability measured for laboratory duplicates (i.e., 40 percent of laboratory duplicates did not meet the quality assurance objectives).

Ribotyping

No major quality control problems were identified with the ribotyping procedure. The following section describes the data assessment procedures, quality control problems, and corrective actions taken for the following quality control elements:

- Completeness
- Methodology
- Blind samples.

Completeness

A total of 552 *E. coli* isolates were obtained from the 80 water samples, which exceeds the objective of 400 isolates for the study. Isolates were obtained from all samples except for the samples collected during the February 9, 2004 base flow sampling event. These missing isolates did not compromise the study objectives because additional isolates were obtained from samples collected two weeks later during the February 25, 2004 base flow sampling event

Methodology

The Institute for Environmental Health reported that the ribotyping analyses were performed in accordance with standard operating procedures and no problems were encountered with those analyses.

A comprehensive independent study of 22 researchers using 12 different microbial source tracking methods was recently conducted by the Southern California Coastal Water Research Project (Griffith et al. 2003). This study included the ribotyping method (Method D) used by the Institute of Environmental Health as part of six microbial source tracking methods that are genotypic-based and require a host origin database (Myoda et al. 2003). Blind-labeled water samples were prepared that each contained between one and three of five possible fecal sources (i.e., sewage, human, dog, cow, and seagull), and were analyzed by each study participant.

This study of microbial source tracking methods found that the ribotyping method (Method D) performed well in several of the evaluation criteria (Myoda et al. 2003). The ribotyping method had high sensitivity rates (i.e., the percentage of time that the source was correctly identified as present in the sample), at 88 percent for human and sewage sources and 81 percent for all sources. The false positive rates for (i.e., the percentage of time the source was incorrectly identified as present in the sample) for the ribotyping method were low, at 17 percent for human and sewage sources and 23 percent for all sources. The ribotyping method correctly identified the dominant source of contamination in 75 percent of all samples.

Several study design issues were identified by Myoda et al. (2003) that might have underestimated the reliability of the methods. Only 50 isolates were examined, representing a small percentage of the bacterial sample population. Also, heterogeneity of sample preparation

and differential bacterial die-off might have resulted in a misrepresentation of the bacteria population. These factors are believed to have only a minor effect on the results of the upper Willapa River MST study because 552 isolates were obtained for the study and all original cultures were prepared on the day of sample collection.

Blind Samples

Fecal coliform bacteria cultures from deer and cow fecal sources were submitted to the Institute of Environmental Health as blind quality control samples. The cultures were prepared by mixing a small amount of feces in sterile water and analyzing the water as routine water samples. A total of six isolates from the deer sample and five isolates from the cow sample were matched to the ribotype database. All 11 isolates obtained from the blind samples were correctly identified.

References

- Griffith, J.F., S.B. Weisberg, and C.D. McGee. 2003. Evaluation of Microbial Source Tracking Methods Using Mixed Fecal Sources in Aqueous Test Samples. *Journal of Water and Health*. 1(4): 141-151.
- Herrera. 2003. Upper Willapa River Microbial Source Tracking Study Sampling and Analysis Plan. Prepared for Cosmopolitan Engineering Group, Tacoma, Washington, and Pacific County Department of Community Development, South Bend, Washington, by Herrera Environmental Consultants, Inc., Seattle, Washington. November 6, 2003.
- Myoda, S.P., C.A. Carson, J.J. Fuhrmann, B-K Hahm, P.G. Hartel, H. Yampara-Iquise, L. Johnson, R.L. Kuntz, C.H. Nakatsu, N.J. Sadowsky, and M. Samadpour. 2003. Comparison of Genotypic-Based microbial Source Tracking Methods Requiring a Host Origin Database. *Journal of Water and Health*. 1(4): 167-180.