Method Comparison

Analysis of Fecal Coliform Samples from Washington State Estuaries

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Method Comparison

Analysis of Fecal Coliform Samples from Washington State Estuaries

by

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Water Resource Inventory Area (WRIA) and 8-digit Hydrologic Unit Code (HUC) numbers for the study area:

WRIAs
- 1 – Nooksack
- 14 – Kennedy-Goldsborough
- 18 – Elwha-Dungeness
- 24 – Willapa

HUC numbers
- 17110002
- 17110019
- 17110020
- 17100106

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Abstract

During 2012 and 2013, the Washington State Department of Ecology conducted an analytical comparison study of two methods used to determine fecal coliform bacteria concentrations in water samples. The purpose of the study was to determine whether a relatively new chromogenic substrate (CS) method, Colilert®-18 (CS-C18), could be used for estuarine water samples in place of the well-established multiple tube fermentation (MTF) method with an A1 growth medium (MTF-A1).

For samples with relatively low bacteria levels, results showed that the experimental CS-C18 method indicated bacteria concentrations significantly lower than the conventional MTF-A1 method.

Recommendations from this study include: (1) continue using established MTF methods in Washington State, (2) conduct future studies to investigate the cause of the method discrepancy, and (3) collect additional comparison samples at a higher concentration range.

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Introduction

High concentrations of fecal coliforms (FC) and other fecal indicator bacteria (FIB) in fresh and marine waters indicate the potential presence of harmful pathogens that pose a public health risk to the people that recreate in these water bodies. In addition, elevated pathogen levels in marine or estuarine waters can accumulate in shellfish tissue, making them unsafe to eat. Consequently, it is important to accurately and consistently monitor FIB in public waters.

A relatively new approach to identifying FIB in water samples relies on the color or fluorescence produced by the reaction between different strains of bacteria and specific enzymes; the methods that utilize this approach are often referred to as enzyme-based or chromogenic substrate (CS) methods. Several studies have demonstrated that CS methods can be comparable to traditional methods, cost-effective, and reproducible (Yakub et al., 2002; Palmer et al., 1996; Redman, 2003).

Both Washington State's water quality standards and the National Shellfish Sanitation Program (NSSP) (FDA, 2009) set limits for bacteria in surface waters based on FC concentrations. The Washington State Department of Health (DOH) implements the NSSP standards and is responsible for evaluating all commercially harvested shellfish areas to determine their suitability for harvest. The Washington State Department of Ecology (Ecology) develops and implements the state's water quality standards.

Ecology conducted this study to compare two different methods used to determine the concentration of fecal coliform bacteria. The goal of this study was to determine whether the Colilert®-18 chromogenic substrate method (CS-C18) (Idexx Laboratories, 2012) could be used as an alternate test procedure by Ecology to quantify FC levels in marine and brackish waters of Washington.

Ecology and DOH staff collected 70 water samples from 4 shellfish growing areas throughout Washington's coastal waters. Field staff immediately split each sample. The DOH laboratory analyzed one portion of the split, using a multiple-tube fermentation (MTF) method (SM9221E2); Ecology's Manchester Environmental Laboratory (MEL) analyzed the other portion, using the CS-C18 method.

Methods

Sampling Locations and Dates

Ecology and DOH staff collected water samples from four different shellfish growing areas (Table 1; Figure 1) in four different regions of Washington's coast. Staff sampled ten routine DOH stations within each growing area, during each event.

Table 1. Sampling locations for the 2012-2013 study.

Region Name	Growing Area Name	County	Latitude[1]	Longitude[1]
North Puget Sound	Drayton Harbor	Whatcom	48.97833	-122.76335
South Puget Sound and Hood Canal	Oakland Bay	Mason	47.22359	-123.06183
Strait of Juan de Fuca	Dungeness Bay	Clallam	48.16053	-123.15686
Pacific Coast, Grays Harbor, and Willapa Bay	North River	Pacific	46.73968	-123.89978

[1] Coordinates for approximate centroid of sampling area.

Field staff completed seven total sampling events between August 2012 and March 2013 (Table 2) with:

- Three sampling events during the 2012 dry season.
- One storm event in December 2012. A storm event, for this study, was defined as a minimum of 0.5" of rain in 24 hours with <0.1" of rain in the preceding 24 hours.
- Three sampling events during the 2012-2013 wet season.

Table 2. Sample event dates for the 2012-2013 study.

Growing Area Name	Sample Event Date	Sample Event Type
Drayton Harbor	August 8, 2012	Dry season
Oakland Bay	August 21, 2012	Dry season
Dungeness Bay	August 27, 2012	Dry season
Oakland Bay	December 17, 2012	Storm event
Dungeness Bay	January 23, 2013	Wet season
Oakland Bay	February 26, 2013	Wet season
North River	March 25, 2013	Wet season

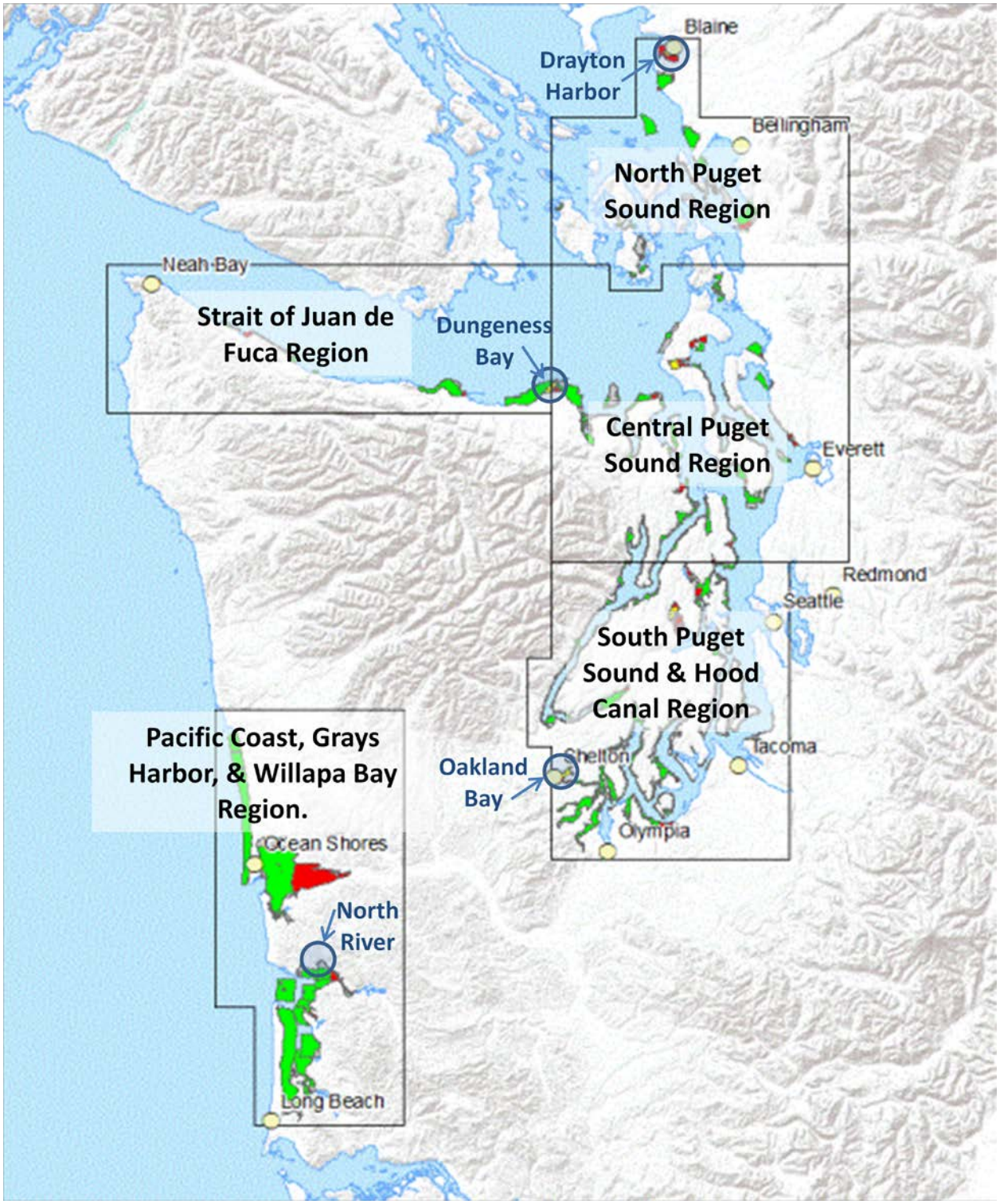

Figure 1. Washington State shellfish growing areas sampled during the 2012-2013 study.

Analytical Methods

DOH analyzed half of the split samples, following standard methods for MTF using an A-1 medium (MTF-A1) (SM9221E2; APHA, 2012). The general procedure involves preparing the A-1 medium, inoculating 15 tubes with 3 different sample dilutions (5 tubes for each dilution), incubating tubes for 3 hours at 35˚C ±0.5˚C, and then incubating tubes for an additional 21 hours at 44.5˚C ±0.2˚C. Gas production within a tube of the A-1 medium indicates a positive result. The analyst then calculates the most-probable number (MPN), using the MPN index tables provided in Standard Methods (APHA, 2012), based on the combination of positive tubes from each dilution.

MEL analyzed the other half of the split samples following the enzyme substrate test for FC (multiple-well procedure) described by Standard Methods (SM9223B; APHA, 2012), the manufacturer's test kit (Idexx, 2012), and the manufacturer's Colilert®-18 FC protocol addendum to test for FC in wastewater. The FC protocol is identical to the total coliform protocol, with the exception that the sealed trays are incubated at a temperature of 44.5°C (±0.2°C), in place of the 35°C incubation temperature.

The general steps in the procedure are:

1. Add contents of one Colilert®-18 snap pack to a 100 mL room temperature water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray®/2000 (QT2000) and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 44.5˚C ±0.2˚C incubator for 18 hours (prewarming to 35˚C is not required). For incubation in a water bath, submerge the QT2000, as is, below the water level using a weighted ring.
5. Read results by comparing the color of each well to a comparator provided by the manufacturer. Count the number of positive wells and refer to the MPN table provided with the QT2000 to obtain the MPN value.

Field Methods

Field staff collected water samples with a sampling wand from the boat deck following the Environmental Assessment Program's Directed Studies Unit Standard Operating Procedures (SOPs) for bacteria (Ward and Mathieu, 2011) and grab sampling (Joy, 2006), as well as DOH Procedure #003 (DOH, 1996).

Ecology or DOH field staff collected samples into sterile 500 mL containers provided by MEL and then *immediately* split the sample three ways as follows:

- Approximately 100 mL sample into a 125 mL bottle provided by DOH for MTF-A1 sample.
- Approximately 200 mL sample into a 250 mL bottle provided by MEL for CS-C18.

- Approximately 200 mL sample retained in the original 500 mL bottle, in case additional sample was needed for quality control procedures, bacterial identification, or alternate analysis methods.

Field staff split and labeled samples and then immediately placed them on ice. Ecology and DOH delivered the samples to their respective laboratories by the morning of the next day and incubated within 24 hours of collection, when possible. For two of the sample events, sampling occurred in the early morning, and the laboratory was unable to incubate samples within 24 hours. The samples were incubated within 30 hours and the associated results were qualified as estimates.

Statistical Methods

Considerations for Censored Data

Data sets with censored data present several issues when we calculate summary statistics and perform statistical tests for significance. Since the true censored values are unknown, often an estimated value is substituted. Commonly used substitution methods include replacing censored data with a zero value (Zero method), half the reporting limit value (Half method), or the full reporting limit value (RL[1] method).

Some statisticians consider these substitution methods to be arbitrary, susceptible to producing large differences in the resulting estimates, and generally not defensible (Helsel and Hirsch, 2002). The Zero and RL substitution methods result in estimates of the mean that are almost certainly either biased low (Zero method) or high (RL method).

Environmental regulatory agencies in Washington use several variations of substitution methods when working specifically with censored bacteria data. Both the Washington Beach Environmental Assessment Communication and Health (BEACH) program and the Shellfish Growing Area Classification program follow National Shellfish Sanitation Program guidelines that state:

> For the purpose of making calculations, fecal coliform counts that signify the upper or lower limit of sensitivity of the test (MPN or ETCP[2]) shall be increased or decreased by one (1) significant figure. Thus, <9.0 becomes 8.9, <17 becomes 16 and >248 becomes 250. (NSSP, 2009)

For MPN data with two significant figures in the result, this approach results in values similar to the RL substitution method. For example, a result of <1.0 becomes 0.9. For bacteria data reported with only one significant figure in the result, this becomes equivalent to the Zero method, if the result is <1.

[1] RL – Reporting Limit
[2] ETCP – Elevated Temperature Controlled Purification

Distributional methods provide another approach to dealing with non-detects, where a distribution is fitted to the uncensored data and then the distribution is used to estimate summary statistics. Popular distributional methods include Maximum Likelihood Estimation and the probability plot method. These methods work well with a large sample size and when the observed data fit a distribution exactly.

Finally non-parametric methods, such as Kaplan-Meier survival curves or Regression on Order Statistics (ROS), can be used to provide estimates of the mean and standard deviation that are not influenced by data distribution or biased in either direction.

To illustrate the difference between the two methods being compared, Ecology calculated summary statistics, using a variety of substitution and non-parametric methods. Ecology calculated Kaplan-Meier and ROS statistics using WQHydro (Aroner, 2011) and then validated by recalculating them using the NADA (Non-detects And Data Analysis for environmental data) package (Lee, 2012) of the open source "R" software (R Core Team, 2012; www.r-project.org).

Paired Prentice-Wilcoxon

Ecology used a nonparametric paired Prentice-Wilcoxon (PPW) test to compare the two analytical methods, using WQHydro for the calculations. The PPW test is designed for use with multiple detection or reporting limits with matched paired data and takes into account the magnitude of difference between the pairs.

Data Quality Objectives and Data Quality

Field

Field replicates for the Colilert method met the precision quality objectives for the study (Table 3). Most of the replicates were pairs of non-detect values and could not be used to calculate the median relative standard deviation (RSD).

Table 3. Field replicate results.

Result	Replicate Result	RSD%
12.2	9.8	15.4%
12	9	20.2%
62	66	4.4%
1	2	47.1%
2	1	47.1%
	Median =	20.2%

Eight additional replicate pairs contained non-detects for one or both values.

Ecology did not meet the completeness objective for the study, where at least 95% of the planned samples were to be collected and analyzed. Ecology planned to collect 100 comparison samples with four wet season, four dry season, and two storm events. Only 70 samples were collected with three wet season, three dry season, and one storm event. Ecology scaled back the project due to several factors, including staff availability, scheduling conflicts, and retirement of MEL's lead microbiologist in October 2012. Even with fewer samples, the sampling events remained well distributed spatially and temporally, and they provided enough paired values for a fairly powerful statistical comparison.

Laboratory

For each sample batch (sample event), MEL analyzed:

- One laboratory blank. All blanks were negative for growth.
- Three control samples:
  - One positive *E. coli* control sample; all samples exhibited growth as expected.
  - One positive non-*E. coli* control sample (*Klebsiella pneumoniae*); all samples were negative for growth, contrary to expected result.
  - One negative (*Pseudomonas aeruginosa*) control sample; all samples were negative for growth as expected.
- One analytical duplicate. All duplicates were within acceptance limits.

MEL also performed coliform bacterial identification following Standard Methods (APHA 2012; SM9225) for 16 isolates extracted from positive (yellow) wells, some fluorescing and some non-fluorescing, (Table 4), using the BBL™ Crystal™ ID panel viewer. Ecology performed the bacterial isolate identification to identify false negatives and potential false positives.

Table 4. Bacteria isolate identification results.

Sample ID	ID
1208080-08 (fluorescing)	*E. coli*
1208080-08 (nonfluorescing)	*Acinetobacter baumannii; less likely Salmonella paratyphi A*
1208082-06 (fluorescing)	*E. coli*
1208082-08 (nonfluorescing)	not identified
1208081-01 (nonfluorescing)	*Shigella sonnei*
1208081-01 (nonfluorescing)	*Vibrio metschnikovii*
1208081-01 (nonfluorescing)	*Pantoea agglomerans*
1208081-02 (fluorescing)	*E. coli*
1208081-11 (fluorescing)	*E. coli*
1208081- *E. coli* (fluorescing)	*E. coli*
1208082-06 (fluorescing)	*E. coli*
1208082-08 (nonfluorescing)	*E. coli*
1303053-02 (fluorescing)	not identified
1303053-08 (nonfluorescing	not identified
1303053-12 (fluorescing)	*Citrobacter freundii or E.coli*
1303053 - *E. coli* (fluorescing)	*E. coli*

The identification method/laboratory was unable to identify three isolates; two were from non-fluorescing wells. MEL correctly identified two *E. coli* positive control isolates as *E. coli* using the test. MEL identified *E. coli* in one non-fluorescing well, indicating a false negative for the test.

MEL also identified four bacterial species not classified as fecal coliforms from the 16 isolates analyzed. Three of these species were identified from one sample (#1208081-01) collected from station #614 in Oakland Bay. The fourth species, *Acinetobacter baumannii*, was isolated from a sample from station #15 in Drayton Harbor. These results do suggest the possibility of a false positive reaction but do not confirm that possibility. A fecal coliform species could have been present in these samples, but not isolated for identification, and still have caused the positive reaction with the Colilert 18 reagent.

Although not of significance to this study, it is interesting to note that the following species of organisms were identified in samples with measureable fecal indicator bacteria, some of which are generally considered pathogenic.

Shigella sonnei is a classic human pathogen, responsible for over two-thirds of the U.S. cases of the infectious disease Shigellosis (CDC, 2014a). *Vibrio metschnikovii* is considered easily identifiable and rarely responsible for human infections or illness (Hansen et al., 1993), particularly compared to other species of *Vibrio*. *Pantoea agglomerans* is an opportunistic human pathogen, fairly ubiquitous in the environment, not easily identifiable, and the most commonly isolated *Pantoea* species from human infections (Delétoile et al., 2009). *Acinetobacter baumannii* can cause a variety of diseases, from pneumonia to blood or wound infections and is the most common cause of *Acinetobacter* infection; however, infections rarely occur outside of healthcare settings (CDC, 2014b).

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Results and Discussion

Of the 70 samples collected for direct comparison, one split sample was damaged in transit to DOH laboratory, leaving 69 samples for comparison. Of these 69 samples:

- For the CS-C18 method (MEL): only 23 samples were within the detection range (67% left-censored data).
- For the MTF-A1 method (DOH): 46 samples were within the detection range (33% left-censored data).
- No "greater-than" results were reported for either method (right-censored data).
- Only 18 samples were within the detection range for both halves of the split sample (uncensored pairs).

Figure 2 contains summary statistics for both methods. For each method, Ecology estimated summary statistics using five different non-detect treatment methods: three substitution methods (Half, Zero, and RL), the Regression on Order Statistics (ROS) method, and the Kaplan-Meier method. The ROS and Kaplan-Meier methods provided an estimate of the mean and standard deviation of the log values. For all methods, the 10th and 90th percentile values were estimated, assuming log-normal distribution, using the following equations:

$$90\text{th percentile} = 10\text{^}(\mu(logFC) + [\sigma(logFC) * 1.2817])$$
$$10\text{th percentile} = 10\text{^}(\mu(logFC) + [\sigma(logFC) * -1.2817])$$

In Washington, state agencies commonly use this calculated 90th percentile value in assessment of shellfish growing areas, swimming beaches, and water quality cleanup plans. Unfortunately, with data sets that contain a large percentage of non-detects and multiple detection limits, there is no true way to know whether the data fit a lognormal−or any other−distribution. Thus the 90th percentile value becomes increasingly uncertain, the higher the percentage of non-detect data.

For the CS-C18 method, the Half, ROS, and Kaplan-Meier methods produced comparable statistics, while the Zero and RL methods produced slightly lower and higher statistics, respectively. Predictably, the Zero method was biased low, given the high percentage of non-detects. The RL method was biased particularly high, given that one batch of samples had nine non-detects with a reporting limit of <10.

For the MTF-A1 method, the RL, ROS, and Kaplan-Meier methods produced comparable statistics while the Zero and Half methods produced slightly lower geometric means and higher 90th percentiles. For all methods, the CS-C18 statistics were lower than the MTF-A1 statistics.

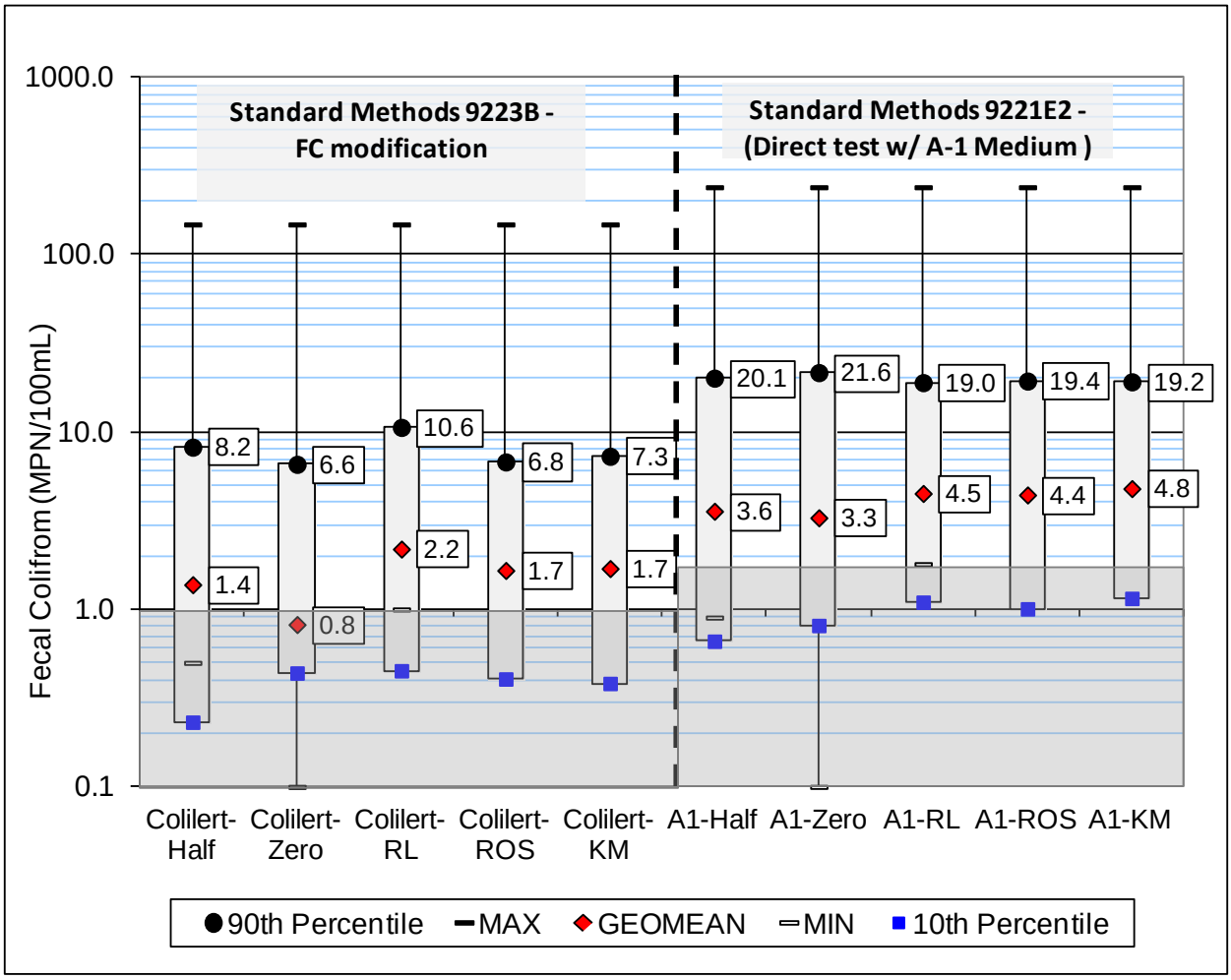

Figure 2. Boxplot of summary statistics for the comparison methods, calculated using varying non-detect treatment methods.

Transparent grey area equals data below the reporting limit.

Ecology also compared the CS-C18 results to the 95% confidence intervals for the MTF-A1 results, using the RL substitution method. 78% of the CS-C18 results fell within the confidence intervals of the MTF-A1 results (Figure 3). Of the results outside the 95% confidence interval, thirteen CS-C18 results fell below the confidence interval. Only two fell above, both of which were <10 results where the upper confidence bound was 6.8 MPN/100 mL. Similar results were obtained using the Half substitution method.

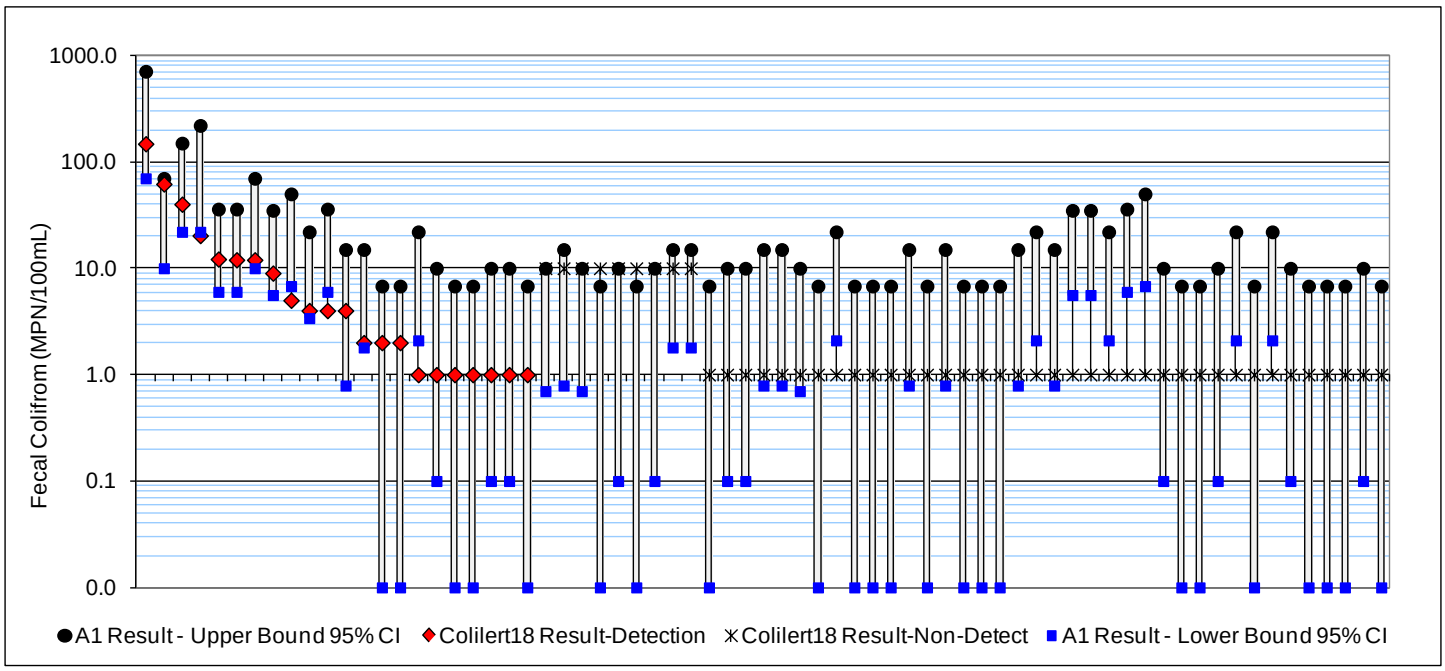

Figure 3. CS-C18 results compared to the 95% confidence intervals of the MTF-A1 results.

Ecology used a non-parametric paired Prentice-Wilcoxon (PPW) test to determine whether or not the CS-C18 method was statistically equivalent to the MTF-A1 method. The null hypothesis for the test was CS-C18 = MTF-A1. A p-value of <0.05 (significance level for this study) indicates that the null hypothesis should be rejected. Table 5 illustrates the results of the PPW test for five different data groups: all paired data, all unqualified paired data (analyzed within holding time), dry season data, wet season data, and uncensored pairs. All five group tests suggested that the null hypothesis should be rejected, providing evidence that supports the alternate hypothesis: that the CS-C18 results were significantly less than the MTF-A1 results.

Table 5. Paired Prentice-Wilcoxon statistical test results.

Data Group	**# of pairs (n)**	**% non-detects (CS-C18 vs. MTF-A1)**	**Z-score**	**p-value**	**Conclusion-** Ho: CS-C18 = MTF-A1 Ha: CS-C18 < MTF-A1
All paired data	69	67% vs. 33%	5.20	0.000	Reject Ho
All data within holding time	50	60% vs. 42%	3.83	0.000	Reject Ho
Dry season pairs	30	87% vs. 33%	3.68	0.000	Reject Ho
Wet season pairs	39	62% vs. 33%	3.83	0.000	Reject Ho
Uncensored pairs	18	n/a	-3.21	0.001	Reject Ho

* Includes storm event samples.

Ecology attempted to find a predictable relationship between the two methods, using the least-squares regression method. The regressions were only performed on the uncensored-pairs sub-group of the data set. The initial regression appeared promising ($r^2 = 0.86$) but was heavily leveraged by one high-concentration data point. With this outlier removed, the relationship decreased drastically ($r^2=0.36$) and the 95% confidence intervals for the regression were very large (Figure 4). Using the logarithms of the results improved the regression relationship; however, the 95% confidence intervals were still rather large (Figure 5). For the purpose of future studies, the regression equation developed from this study should not be used to convert CS-C18 values for comparison to MTF-A1 results.

Results of this study suggest that, at relatively low concentrations (< 100 MPN/100 mL), the CS-C18 method consistently produced lower FC results, compared to the MTF-A1 method. Although the study results only included a lower range of concentrations, these fecal coliform levels are highly relevant for estuarine waters, given that the chronic and acute standards for shellfish growing areas in Washington are set at 14 and 43 MPN/100 mL, respectively.

Several possible theories could explain the difference between the two methods:

- The CS-C18 method may have failed to detect or accurately quantify *Klebsiella* species within the samples.
  - Olstadt et al. (2007) found that the CS-C18 method failed to identify the presence of *Klebsiella* at low spike levels, at one out of three sample sites, although the test was successful at higher spike levels for this site.
  - For this study, all seven *Klebsiella Pneumoniae* control samples submitted for CS-C18 analysis tested negative for growth. These control samples were also relatively low concentration, containing between 1-50 cfu/sample.

- The CS-C18 method may have failed to detect or accurately quantify all *E. coli* strains within the samples.
  - Ecology observed one potential false negative in the results for this study.
  - One comparison study found that the CS-C18 method produced lower *E.coli* counts and higher false-negative rates (11%), compared to the traditional MTF and membrane filtration (MF) methods (Schets et al., 2002).
  - In contrast, several studies have reported relatively low false negative rates such as 3.5% (Chao et al., 2004) and 7% (Warden et al., 2011).

- The A1 medium may have produced a higher rate of false positives.
  - No direct evidence was observed to support this theory. However, Ecology did not investigate false positives or negatives in the MTF-A1 samples, because the method is well established, and any method performance evaluation was outside the scope of this study.
  - In several studies of samples from biosolids, the A1 medium produced a higher rate of false positives, as compared to lauryl-tryptase broth (Baker et al., 2005; EPA, 2008).

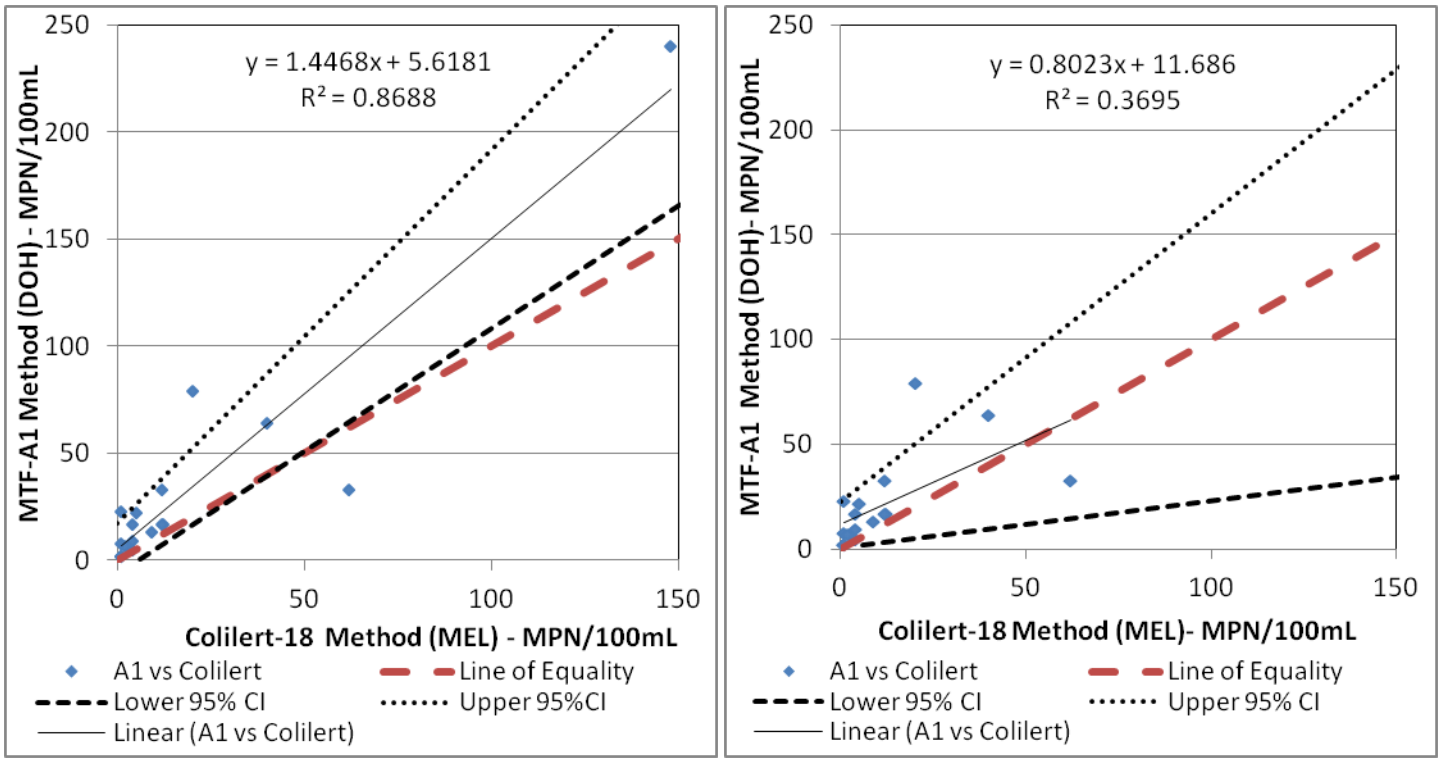

Figure 4. Regression equations for the comparison methods, with (left) and without (right) a leverage outlier.

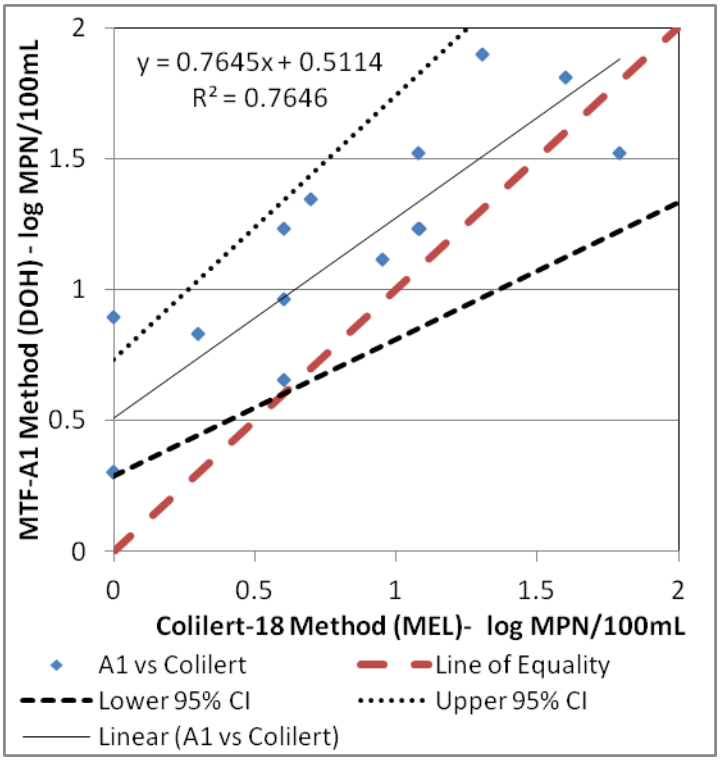

Figure 5. Regression equation, using the log of results, for the comparison methods, with leverage outlier removed.

Conclusions and Recommendations

Conclusions

Results of this 2012-13 study support the following conclusions:

- For estuarine waters of Washington State, with relatively low bacteria concentrations, the Colilert®-18 /Quanti-Tray®-2000 chromogenic substrate method (CS-C18) for fecal coliform quantification did not prove comparable to the currently practiced A1-medium, multiple-tube-fermentation method (MTF-A1).
- The CS-C18 method produced results that were significantly lower than the MTF-A1 method, at low concentrations, and the method had a higher percentage of non-detect results (CS-C18 = 67% non-detects; MTF-A1 = 33% non-detects).
- Ecology did not identify a strong, predictable relationship between the two methods, based on the results of this study.

Recommendations

Results of this 2012-13 study support the following recommendations:

- Washington State regulatory agencies should continue to use the approved MTF methods currently practiced for fecal coliform quantification in estuarine and marine waters.
- The discrepancy between the two methods warrants further investigation. Future studies should focus on the CS-C18 method's ability to enumerate *Klebsiella* species, different strains of *E. coli*, and injured organisms.
- Additional comparison samples are needed from brackish waters with higher concentrations of fecal coliforms, such as tidally-influenced streams or sloughs in agricultural or urban watersheds.

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Appendices

Appendix A. Results Table

Location	Sample Date	Sample Time	Sample ID	Colilert-18 Result	Qualifier	Colilert-18 Replicate Result	Replicate Qualifier	MTF-A1 Result	Qualifier
DH-15	8/8/2012	10:25:00	1208080-08	148	J			240	
DH-8	8/8/2012	10:35:00	1208080-05	10	UJ	10	UJ	4	
DH-6	8/8/2012	10:40:00	1208080-04	10	UJ			4.5	
DH-4	8/8/2012	10:42:00	1208080-02	10	UJ			4	
DH-315	8/8/2012	11:15:00	1208080-10	10	UJ			1.8	U
DH-3	8/8/2012	11:20:00	1208080-01	10	UJ	10	UJ	2	
DH-314	8/8/2012	11:24:00	1208080-09	10	UJ			1.8	U
DH-12	8/8/2012	11:30:00	1208080-07	10	UJ			2	
DH-5	8/8/2012	11:35:00	1208080-03	10	UJ			6.8	
DH-11	8/8/2012	11:37:00	1208080-06	10	UJ			6.8	
Oak-128	8/21/2012	9:49:00	1208081-05	1	U			1.8	U
Oak-639	8/21/2012	9:52:00	1208081-06	1	U			2	
Oak-615	8/21/2012	10:01:00	1208081-07	1	U			2	
Oak-614	8/21/2012	10:27:00	1208081-01	12.2		9.8		17	
Oak-129	8/21/2012	10:34:00	1208081-02	1				7.8	
Oak-758	8/21/2012	10:40:00	1208081-03	1	U			4.5	
Oak-127	8/21/2012	10:46:00	1208081-04	1	U			4.5	
Oak-114	8/21/2012	11:10:00	1208081-09	1	U			4	
Oak-124	8/21/2012	11:15:00	1208081-10	1	U	1	U	1.8	U
Oak-115	8/21/2012	11:26:00	1208081-08	1	U			7.8	
Dun-115	8/27/2012	12:11:00	1208082-01	1	U			1.8	U
Dun-114	8/27/2012	12:17:00	1208082-02	1	U	1	U	1.8	U
Dun-103	8/27/2012	12:26:00	1208082-03	1	U	1	U	1.8	U
Dun-113	8/27/2012	12:31:00	1208082-04	1	U			4.5	
Dun-104	8/27/2012	12:34:00	1208082-06	20.3				79	
Dun-105	8/27/2012	12:39:00	1208082-05	1	U			1.8	U
Dun-108	8/27/2012	12:44:00	1208082-07	1	U			4.5	
Dun-110	8/27/2012	12:48:00	1208082-08	1				2	
Dun-111	8/27/2012	12:53:00	1208082-09	1	U			1.8	U
Dun-112	8/27/2012	12:58:00	1208082-10	1	U			1.8	U
Oak-114	12/17/2012	9:39:00	1212028-09	4				9.2	
Oak-124	12/17/2012	9:46:00	1212028-10	1	U			1.8	U
Oak-115	12/17/2012	10:00:00	1212028-08	9				13	
Oak-128	12/17/2012	10:15:00	1212028-05	4				17	
Oak-639	12/17/2012	10:20:00	1212028-06	12				17	
Oak-615	12/17/2012	10:30:00	1212028-07	12		9		33	

Location	Sample Date	Sample Time	Sample ID	Colilert-18 Result	Qualifier	Colilert-18 Replicate Result	Replicate Qualifier	MTF-A1 Result	Qualifier
Oak-127	12/17/2012	10:50:00	1212028-04	4				4.5	
Oak-758	12/17/2012	10:54:00	1212028-03	5				22	
Oak-129	12/17/2012	11:03:00	1212028-02	62		66		33	
Oak-614	12/17/2012	11:12:00	1212028-01	40				64	
Dun-115	1/23/2013	8:44:00	1301038-01	1	UJ				
Dun-114	1/23/2013	8:51:00	1301038-02	1	UJ	2	J	4.5	
Dun-103	1/23/2013	8:59:00	1301038-03	1	UJ			7.8	
Dun-113	1/23/2013	9:05:00	1301038-04	1	UJ			4.5	
Dun-104	1/23/2013	9:08:00	1301038-06	1	UJ			13	
Dun-105	1/23/2013	9:14:00	1301038-05	2	J			6.8	
Dun-108	1/23/2013	9:19:00	1301038-07	1	UJ			13	
Dun-110	1/23/2013	9:23:00	1301038-08	1	UJ			7.8	
Dun-111	1/23/2013	9:28:00	1301038-09	1	UJ			17	
Dun-112	1/23/2013	9:32:00	1301038-10	1	J			23	
Oak-127	2/26/2013	8:49:00	1302045-04	2				1.8	U
Oak-758	2/26/2013	8:57:00	1302045-03	1				1.8	U
Oak-129	2/26/2013	9:02:00	1302045-02	1				1.8	U
Oak-614	2/26/2013	9:06:00	1302045-01	1	U			2	
Oak-128	2/26/2013	9:34:00	1302045-05	1	U			1.8	U
Oak-639	2/26/2013	9:41:00	1302045-06	2		1		1.8	U
Oak-615	2/26/2013	9:46:00	1302045-07	1				2	
Oak-115	2/26/2013	10:05:00	1302045-08	1	U	1		1.8	U
Oak-114	2/26/2013	10:10:00	1302045-09	1	U			2	
Oak-124	2/26/2013	10:16:00	1302045-10	1	U			7.8	
NR-74	3/25/2013	11:50:00	1303053-01	1	U			1.8	U
NR-69	3/25/2013	11:54:00	1303053-02	1				2	
NR-64	3/25/2013	11:59:00	1303053-05	1	U			7.8	
NR-66	3/25/2013	12:08:00	1303053-03	1	U			2	
NR-68	3/25/2013	12:11:00	1303053-04	1	U	1	U	1.8	U
NR-60	3/25/2013	12:21:00	1303053-06	1	U			1.8	U
NR-67	3/25/2013	12:24:00	1303053-07	1	U			1.8	U
NR-61	3/25/2013	12:31:00	1303053-08	1				1.8	U
NR-63	3/25/2013	12:38:00	1303053-10	1	U			2	
NR-62	3/25/2013	12:42:00	1303053-09	1	U	1		1.8	U

J = Analyte was positively identified. The reported result is an estimate.
U = Analyte was not detected at or above the reported result.
UJ = Analyte was not detected at or above the reported estimate

Appendix B. Glossary, Acronyms, and Abbreviations

Glossary

A1 medium (A1): A liquid culture medium used in the detection of fecal coliforms in foods, treated wastewater, and marine waters using the most-probable number method for enumeration.

Colilert-18® chromogenic substrate method (CS-C18): An analytical method used to test for the presence of Total Coliforms or *E. coli* based on the color or fluorescence produced by the reaction between different strains of bacteria and specific enzymes. The method is described in Standard Methods under section SM9223B (APHA, 2012). Used in combination with a Quanti-Tray or Quanti-Tray/2000, the Colilert-18 reagent can be used to enumerate bacteria concentrations using a multiple-well, most-probable number system. A modification to the incubation temperature (44.5°C in place of 35°C) is recommended by the manufacturer to determine Fecal Coliform concentrations.

Geometric mean: A mathematical expression of the central tendency (an average) of multiple sample values. A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a straight average (arithmetic mean) were calculated. This is helpful when analyzing bacteria concentrations, because levels may vary anywhere from 10 to 10,000 fold over a given period. The calculation is performed by either: (1) taking the nth root of a product of n factors, or (2) taking the antilogarithm of the arithmetic mean of the logarithms of the individual values.

Most-probable number (MPN): A method used to estimate the concentration of samples, typically microbiological, by diluting the sample multiple times, replicating each dilution, and then testing for presence/absence in each sub-sample/dilution. The combination of positive and negative reactions for each dilution can be used to estimate the concentration in the original sample using statistical probability.

Pathogen: Disease-causing microorganisms such as bacteria, protozoa, viruses.

Quanti-Tray®-2000: A proprietary multiple-well tray that is used to enumerate bacteria samples inoculated with a Colilert® reagent. The sample is poured into the tray, passed through a mechanical sealing device, and then incubated at a given temperature for a given amount of time. Positive reactions within the wells are used to estimate bacteria concentrations using the most-probable number method.

90th percentile: A statistical number obtained from a distribution of a data set, above which 10% of the data exists and below which 90% of the data exists.

Acronyms and Abbreviations

C18	Colilert-18® reagent
CS	Chromogenic substrate
DOH	Washington State Department of Health
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
FC	Fecal coliform
FIB	Fecal indicator bacteria
GIS	Geographic Information System software
MEL	Manchester Environmental Laboratory
MPN	Most-probable number
MTF	Multiple-tube Fermentation
QT-2000	Quanti-tray 2000® multiple well tray (see Glossary for detail)
RL	Reporting limit
RSD	Relative standard deviation
SOP	Standard operating procedures
WRIA	Water Resource Inventory Area

Units of Measurement

°C	degrees centigrade
cfu	colony forming units
mL	milliliters
MPN	most-probable number