Alternative Bacteria Source Identification using Colilert®/Quanti-Tray 2000 Test Method in Irrigated Agricultural Watersheds

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July 8, 2011

This report is the final requirement of Contract C0900151 between the Washington Department of Ecology and the Roza-Sunnyside Board of Joint Control in compliance with the Environmental Protection Agency demonstration project: EPA-R10-08-OWW-WU.

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Abstract

The Washington State Department of Ecology (Ecology) received grant funds for Innovative TMDL Development Projects from the United States Environmental Protection Agency (EPA), Region 10. This demonstration project is one of several Ecology projects that received such funding. The goals of this project were: (1) to allow extensive monitoring of bacterial pollution throughout a large agricultural watershed by a staff-limited and space-limited local agency; (2) to determine if the use of *E. coli* monitoring can be an acceptable surrogate for FC bacteria in watersheds; and (3) to accelerate the development of required bacterial TMDLs and, thereby, accelerate the improvement of an agricultural watershed's water quality.

The Sulphur Creek Wasteway (SCW) watershed is located in south-central Washington State and is divided into seven sub-basins based on its various tributaries (Figure 1). The Roza-Sunnyside Board of Joint Control (RSBOJC) has conducted extensive FC bacteria monitoring throughout all of the sub-basins of the SCW. All but one of the sub-basins had significantly greater FC bacteria densities during the *irrigation season* "critical condition", which suggests a predominance of "diffuse non-point sources" of bacteria. The lower sub-basin JD 33.4, however, showed *year-round* high bacteria densities, which suggests that the predominant bacteria sources are "discrete point sources".

Several water samples needed to be analyzed throughout the lower sub-basin JD 33.4 in order to locate more precisely the suspected point sources of bacteria. However, the RSBOJC, like many rural agencies, has limited laboratory staff and floor-space. This demonstration project specifically utilized alternative technologies, Colilert®/Quanti-Tray 2000 (IDEXX methodology), for the analysis of *E. coli* as a substitute for the more labor-intensive membrane filtration (MF) analysis for FC bacteria. The EPA officially approved the Colilert® method for testing ambient waters on July 21, 2003.

The alternative technologies allowed the RSBOJC's 2-person laboratory to analyze 200+ additional water samples, without the need to increase laboratory staff or floor-space. This demonstration project successfully determined that the greatest sources of FC pollution in the lower sub-basin JD 33.4 are due to discrete point sources, as was hypothesized. The additional samples helped identify and locate previously unknown inputs of sanitary sewage within the boundaries of the city of Sunnyside and which were quickly repaired.

It is the authors' opinion that the three goals of this demonstration project were successfully achieved.

Background

General Description

The Sulphur Creek Wasteway (SCW) watershed is located in Yakima County within the State of Washington (State), approximately 35 miles southeast of the city of Yakima and 45 miles northwest of the Tri-Cities area (Richland/Kennewick/Pasco). The SCW is situated within Water Resource Inventory Area (WRIA) 37 with its center-point at Latitude: 46.27861 and Longitude: -120.00056. The watershed contains the majority of the city of Sunnyside and a vast amount of surrounding irrigated agricultural lands, which includes 40 concentrated animal feeding operations (CAFOs).

The 96,000-acre (150 square miles) watershed lies in the Yakima River valley and is bounded by the Horse Heaven Hills anticline to the south and the Rattlesnake Hills anticline to the north. The soil is deep, well-drained, fertile silt loam (Zuroske, 2004). The natural vegetation of the SCW watershed is categorized as shrub-steppe, consisting of various sagebrushes and bunchgrasses. However, when the land is cleared and irrigation water is applied, an unlimited variety of crops can be grown in the SCW watershed.

The climate of the area is generally characterized as mild and dry. Summer air temperatures range from 85 to over 100 degrees Fahrenheit. Winters are generally cool with air temperatures often falling below freezing from November through January, sometimes reaching 20 degrees below zero Fahrenheit. Annual snowfall is light and averages 10 to 15 inches. The SCW watershed receives 6 to 8 inches of annual precipitation, with the principle growing season (June, July and August) receiving less than one inch of measurable precipitation. The general weather conditions throughout the project reflected the typical year-round conditions of the area.

The SCW is a man-made drainage canal (7.5 miles in length) that ultimately collects all of the irrigation return flows, municipal stormwater, and some State-permitted municipal and industrial discharges that occur within the watershed. It was constructed in 1908-1910 and serves as the Sunnyside Division's principal man-made drainage canal. The drainage canal system is owned by the United States Bureau of Reclamation and is part of that agency's Yakima Project.

Land-uses

In addition to approximately 1,500 acres of irrigated agricultural lands, the lower sub-basin JD 33.4 includes: seven CAFOs, urban residential and industrial sections, "hobby farms" and several rural residences. The drainage from the sub-basin is a complex combination of runoff from irrigated agriculture and rural properties, stormwater flows from the central business district of the city of Sunnyside, effluent discharges from the city's wastewater treatment facility (Publicly-owned Treatment Works; AKA: POTW), and discharges from the Port of Sunnyside industrial property.

Bacterial Problem

Analysis of water quality data from 1968 through 1985 by the United States Geological Survey (USGS) found that the SCW watershed fecal coliform (FC) bacteria densities were "among the largest observed throughout the Yakima River Basin" (Embry, 1992). Morace et al. (1999) determined that agricultural practices caused increased fecal-indicator bacteria in streams that receive irrigation return flows throughout the lower Yakima River basin. Fuhrer et al. (2004) suggested that the excessive FC bacteria densities in the SCW were related to the watershed's high density of livestock. The Washington State Department of Ecology (Ecology) water quality monitoring near the mouth of the SCW found FC geometric means of 1,237 cfu/100mL and 1,437 cfu/100mL during 1993 and 1994, respectively. The SCW has historically exceeded the State's FC surface water quality dual criterion of 200/400 cfu/100mL (geometric mean/90% value) for that specific waterway. All of the other waterways in the watershed are required to comply with the State's FC surface water quality dual criteria of 100/200 cfu/100mL

Figure 1 shows the seven sub-basins composing the SCW watershed.

Figure 1. Sub-basins within the Sulphur Creek Wasteway Watershed

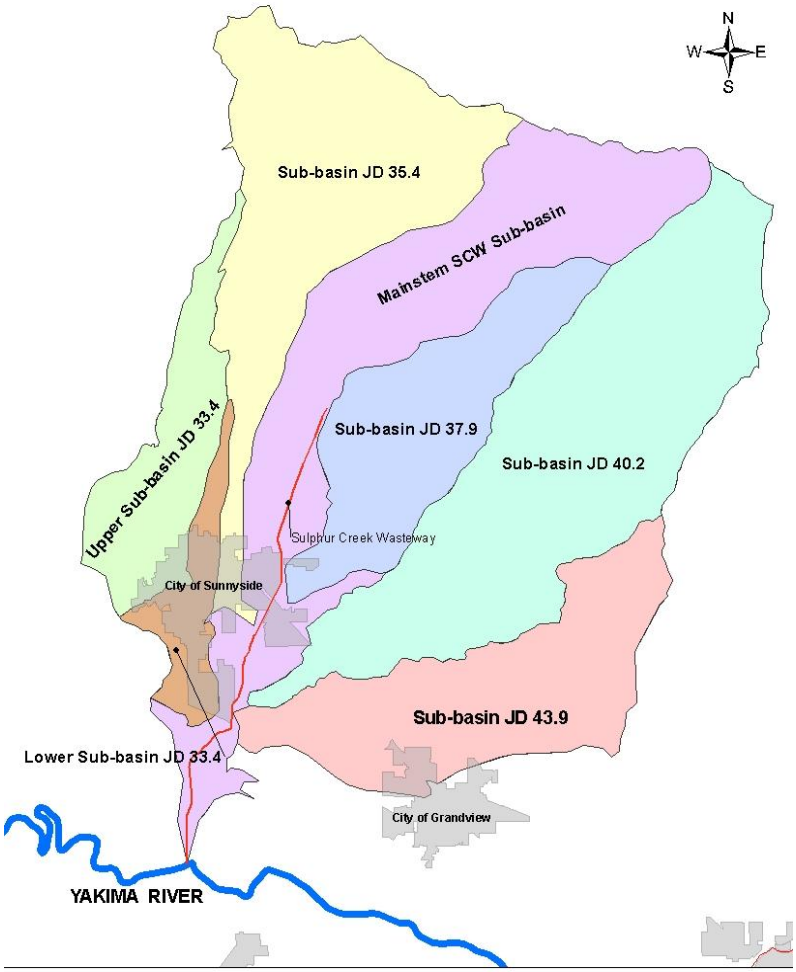


Table 1 details the annual geometric mean FC bacteria densities found during the irrigation season (April 1 – October 31) at each of the seven SCW sub-basins from 1999 through 2005.

Table 1. Irrigation Season Geometric Mean FC Bacteria Densities in SCW Sub-basins, 1999-2005

Sub-basin # → / Year of Sampling ↓	Lower JD 33.4	Upper JD 33.4	JD 43.9	JD 40.2	JD 35.4	JD 37.9	Mainstem SCW
1999	3,726	1,092	1,130	1,436	508	359	800
2000	815	715	801	1,181	251	353	457
2001	507	344	610	860	134	221	361
2002	1,540	No Data	301	580	172	196	410
2003	775	243	301	592	160	249	285
2004	1,003	319	451	633	250	136	364
2005	1,327	309	333	791	218	299	369

Table 2 details the annual geometric mean FC bacteria densities found during the non-irrigation season (November 1 – March 31) at each of the seven SCW sub-basins from 1999 through 2005.

Table 2. Non-irrigation Season Geometric Mean FC Bacteria Densities in SCW Sub-basins, 1999-2005

Sub-basin # → / Year of Sampling ↓	Lower JD 33.4	Upper JD 33.4	JD 43.9	JD 40.2	JD 35.4	JD 37.9	Mainstem SCW
1999	833	370	314	272	200	120	278
2000	1,937	251	164	129	100	72	383
2001	3,047	340	256	193	135	43	333
2002	1,117	No Data	209	268	520	247	355
2003	1,169	61	186	121	62	28	329
2004	1,350	112	135	190	433	83	283
2005	3,600	70	170	140	136	62	632

During both the irrigation and non-irrigation seasons, the greatest FC densities in the SCW watershed occurred within the lower sub-basin JD 33.4. Coincidentally, it is the smallest of the seven sub-basins and includes the largest percentage of municipal/urban areas. All of the other SCW sub-basins have significantly lower FC pollution during the agricultural non-irrigation season. In fact, those sub-basins' non-irrigation FC densities averaged only 59% of their respective irrigation season densities. Whereas, the non-irrigation season bacteria densities in the lower sub-basin JD 33.4 averaged 213% of its irrigation season densities. The latter increase in bacteria densities is in part a consequence of less dilution due to a 28.2% reduction in that sub-basin's flows during the non-irrigation season.

Ecology hypothesized that the year-round occurrence of high FC bacteria densities indicates that the predominant sources of bacterial pollution in the lower sub-basin JD 33.4 are probably "discrete point sources" rather than "diffuse non-point sources". For purposes of this demonstration project, the terms "point source" and "non-point source" have no relationship to any State or federal permit system or requirement. They only refer to whether any specific discharge is physically "discrete" (i.e. a pipe) or "diffuse" (i.e. agricultural return flow).

Project Components

General Description

Ecology received grant funds (EPA-R10-08-OWW-WU) for Innovative TMDL Development Projects from the United States Environmental Protection Agency (EPA), Region 10. Ecology subsequently contracted with the Roza-Sunnyside Board of Joint Control (RSBOJC) to conduct a demonstration project (Contract #C0900151) with the following goals: (1) to allow extensive monitoring of bacterial pollution throughout a large agricultural watershed by a staff-limited and space-limited local agency; (2) to determine if the use of *E. coli* monitoring can be an acceptable surrogate for FC bacteria in watersheds; and (3) to accelerate the development of required bacterial TMDLs and, thereby, accelerate the improvement of an agricultural watershed's water.

This demonstration project required the collection of a minimum of eight water samples at each of twenty-five different sampling sites (total of 200 samples), all of which are located throughout the lower sub-basin JD 33.4 (Figure 2). Thirty of those samples were required to be either split in the field or in the laboratory for quality assurance (QA) purposes. Additional samples were also analyzed using membrane filtration for FC bacteria and for *E. coli*, for method comparison.

Under normal workload conditions, the RSBOJC's small water quality laboratory would not be able to collect and analyze all of the additional samples required by this demonstration project if its present membrane filtration (MF) methodology were to be utilized. This demonstration project was proposed specifically to utilize a more rapid alternative bacteria analysis methodology that has already been approved by the EPA. The alternative methodology is a combination of the Colilert® and Quanti-Tray 2000[1] technologies, which will be herein referred to as the IDEXX methodology.

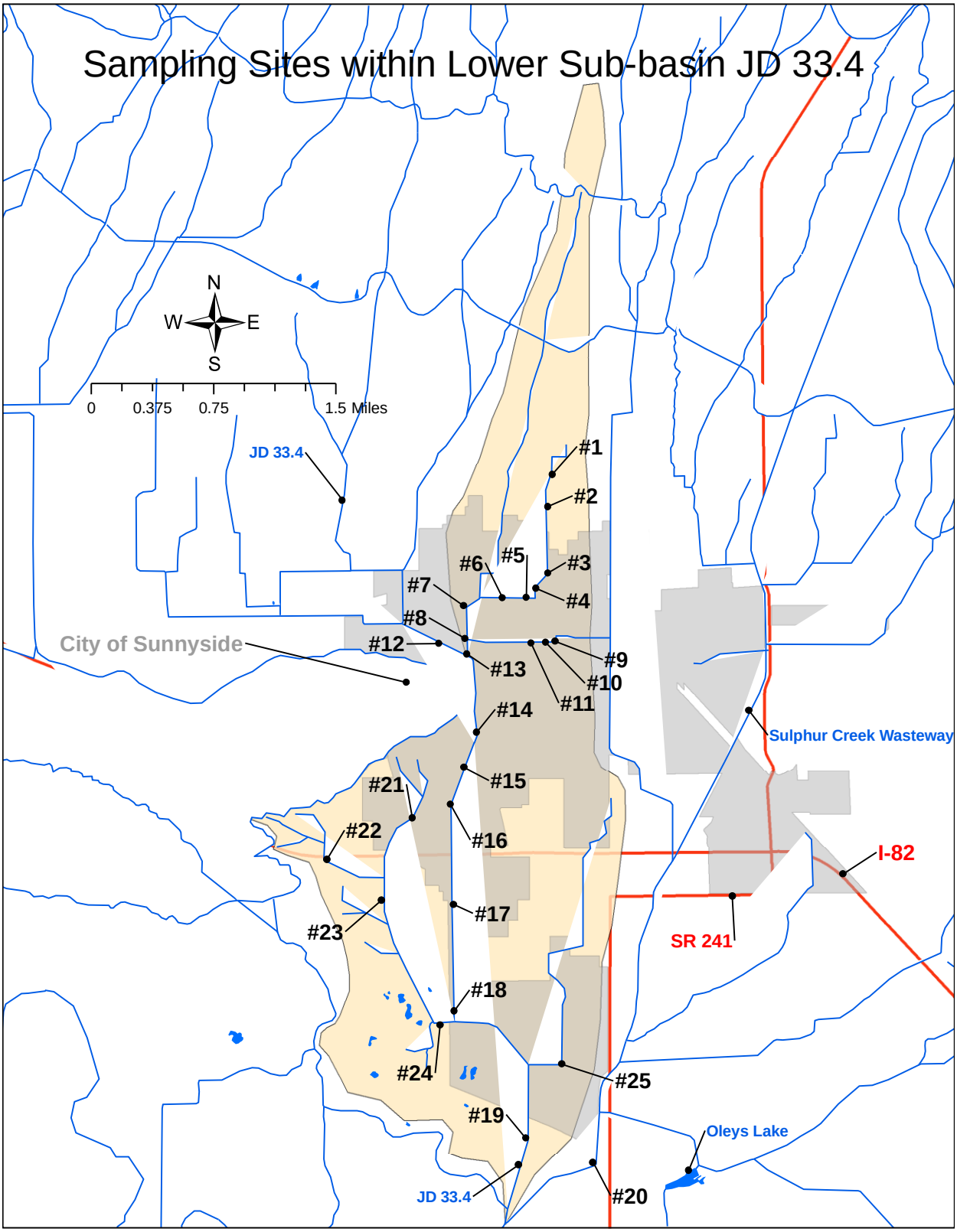
E. coli as a Surrogate for FC Indicator of Bacterial Pollution

The use of *E. coli* as a surrogate for FC bacteria is logical as the prior species typically composes 95-98% of the latter bacterial group in freshwater samples collected throughout the State (Hicks, 2002). The RSBOJC specifically analyzed water samples collected throughout the SCW (2002-2006) for both *E. coli* and FC bacteria densities using MF methodologies. Analysis of that data (n = 285) indicated a geometric mean *E. coli*/FC ratio of 1.18. A ratio of 1.00 would indicate equivalent densities.

In addition, several researchers have previously determined that the IDEXX methodology produces statistically equivalent results to those obtained by the MF methodology (Lewis and Mak, 1989; Olson et al., 1991; Clark, 1991; Eckner, 1998; Chao et al., 2004, Buckalew, 2005). The use of the IDEXX methodology was suggested as a better choice for smaller water quality laboratories than the typical MF methodology, since it requires substantially less "hands-on" time to run. The ability to conduct a greater number of analyses would hypothetically accelerate the development of required bacterial TMDLs and, thereby, accelerate the improvement of an agricultural watershed's water quality.

[1] Colilert® and Quanti-Tray 2000 are registered trademarks of the IDEXX Laboratories, Westbrook, Maine.

Figure 2. Sampling Sites within the Lower Sub-basin JD 33.4



IDEXX Methodology

The IDEXX methodology is used for the detection, confirmation and enumeration of *E. coli* in surface waters within 28 hours. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When *E. coli* metabolize the nutrient indicator (MUG) by the enzyme ß-D-glucuronidase, a fluorescent compound (MUF) is released. EPA officially approved[2] the Colilert® technology for testing ambient waters, which is the same as enzyme substrate test as SM 9223B.

100 milliliters of each water sample is added to a pre-sterilized vial. Each vial then receives the contents of one Colilert®-MUG substrate packet. The vial is quickly resealed, shaken until contents are dissolved and then poured into a Quanti-Tray 2000 enumeration tray. The Quanti-Tray is then sealed and placed into an incubator at 35°C for 24 hours. An ultraviolet light is used to make the actual counts of the fluorescent yellow *E. coli* colonies. Each Quanti-Tray contains 97 wells of two different sizes to achieve bacterial counts of 1 to 2,419 MPN/100mL, with a 95% confidence limit.

Objectives and Desired Outcomes

This demonstration project's goals are:

- To allow extensive monitoring of bacterial pollution throughout a large agricultural watershed by a staff-limited and space-limited local agency;
- To determine if the use of *E. coli* monitoring can be an acceptable surrogate for FC bacteria; and
- To accelerate the development of required bacteria TMDLs and, thereby, accelerate the improvement of an agricultural watershed's water quality.

This demonstration project's anticipated outputs are:

- An extensive set of *E. coli* monitoring data which will allow Ecology to locate suspected point sources of bacteria pollution within the lower sub-basin JD 33.4;
- A final project report that will be disseminated to Ecology and the various conservation districts throughout the State and that will also be posted on the RSBOJC internet website for educational purposes; and
- Obtaining State-accreditation for the IDEXX methodology at the RSBOJC laboratory.

Results of IDEXX Methodology Quality Assurance Testing

Sampling, laboratory analysis, and data evaluation steps have several sources of error that should be addressed by data quality objectives. The primary determinants for data QA for this demonstration project are accuracy and precision. QA testing was only conducted on the IDEXX methodology as the RSBOJC laboratory already performs QA for its MF methodologies for both *E. coli* and FC bacteria in order to maintain State accreditation.

[2] Published in Federal Register dated July 21, 2003.

Accuracy:

Accuracy measures how close laboratory results are to a true or expected value. It is usually measured by analyzing a sample "spiked" with a known concentration, or by measuring directly a known amount if "spikes" are not applicable. The accuracy of bacteria samples are normally handled differently from chemical pollution as a bacterial "spike" is not typically available. Thus, the MQO established in this project's QAPP was stated as "<25% Relative Percent Difference (RPD)".

It was not known until after the project had started that bacterial "spikes" were indeed available. MicroBiologics® E^{power} pellets (Lot # 483711) were utilized as a known amount (7,800 cfu/100mL) of *E. coli* bacteria. The pellets are lyophilized, quantitative microorganism preparations used in industrial laboratories for QA purposes. One pellet was dissolved in 100mL of a 7.2 buffer solution (according to manufacturer's instructions) and then split into 10 replicate samples.

Due to the inherent variability of bacteria measurements, Ecology later suggested that "25% to 175% Recovery" would be the more appropriate MQO. % Recovery = ((Xs – Xo)/Xo) x 100%, where: Xs = spike sample result, and Xo = original sample amount. The calculated % Recoveries ranged from 118% to 181.2% (Appendix A). The average % Recovery was 143.6%.

Another accuracy test was also performed using Environmental Resource Associates (ERA) WP WasteWatR™ Coliform MicrobE™ product which similarly contains lyophilized *E. coli* bacteria. The IDEXX methodology using the "spike" of 859 MPN/100mL recovered 866.4 MPN/100mL which was within the manufacturer's acceptable limits of recovery (393 – 1,880 MPN/100mL). The % Recovery using this product was 100.9%.

The difference in % Recoveries between the two commercial products may have to do with the amount of *E. coli* contained within the products. The latter product which contains significantly less bacteria gave the best recovery. This may indicate that the IDEXX methodology is more precise for lower concentrations (<5,000 cfu/100mL) of *E. coli*.

Precision:

Precision, expressed as RPD and obtained from analysis of field sample splits, is a measure of the reproducibility of a result while subject to random error. Random error may occur during sample handling, preservation, storage, and analysis stages. Precision was estimated by the RPD of the actual data obtained from analysis of the field-split samples (Appendix B). RPD = ((S – D)/((S+D)/2)) x 100%, where: S = analytical result of sample of origin, and D = analytical result of the duplicate sample.

The original precision RPD was listed in the QAPP as "<25% RPD of log-normalized data". However, subsequent revision by Ecology's Environmental Assessment Program (EAP) indicated that "<40% RPD of actual data" would be more appropriate. The calculated RPDs of twenty paired field-split analyses ranged from 0.0% to 51.7% (Appendix B). The average RPD was 17.7%.

Results of Methodology Comparison Testing

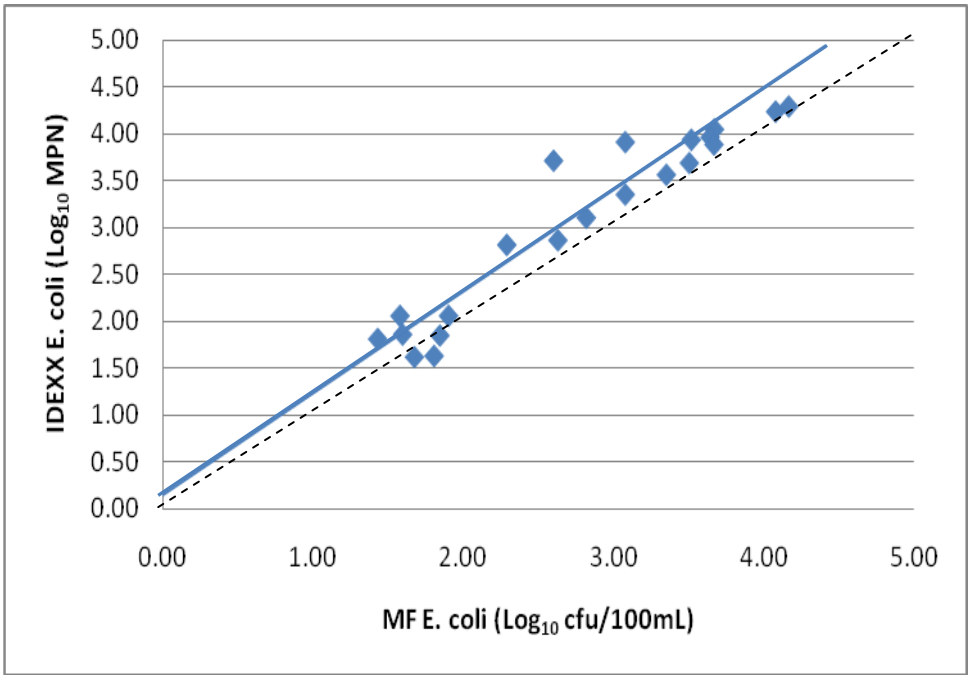
E. coli densities using both the IDEXX and MF (m-ColiBlue24®) methodologies:

This demonstration project analyzed 21 split samples for a comparison between the IDEXX (Colilert®/Quanti-Tray 2000) and MF (m-ColiBlue24®)[3] methodologies. A simple regression analysis of the log-normalized data from both methodologies is presented in Figure 3.

[3] m-ColiBlue24® is a registered trademark of the Hach Company, Loveland, Colorado.

The resultant Pearson correlation coefficient (R) of 0.957 ($p < 0.0000$) indicates a very strong significant correlation between the two methodologies. The IDEXX methodology data is expressed in MPN; while the MF methodology data is expressed in cfu/100mL. The formula for the regression line is: Log_{10} IDEXX *E. coli* = 0.215683 + 1.03*Log_{10} MF *E. coli*.

Figure 3. Log_{10} IDEXX *E. coli* Densities vs. Log_{10} MF *E. coli* Densities

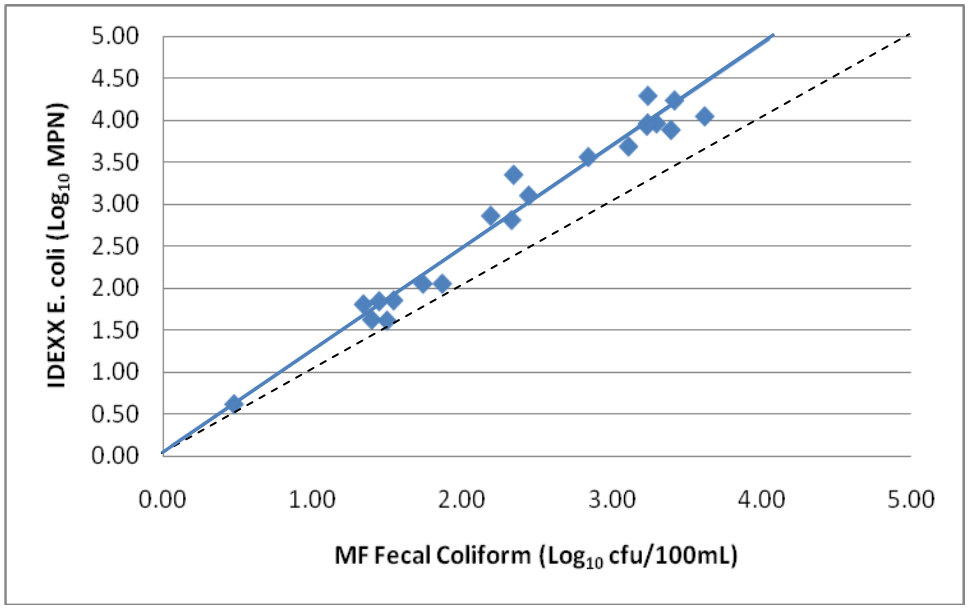


The *E. coli* densities obtained from the IDEXX methodology were generally greater than the densities obtained from the MF (m-ColiBlue24® media) methodology. The geometric mean % Error between the actual bacteria density data of the two methodologies was 41.4%. This should be interpreted as the IDEXX methodology enumerating significantly greater *E. coli* densities than the MF (m-ColiBlue24® media) methodology.

E. coli densities using the IDEXX methodology vs FC densities using the MF (m-FC media with rosolic acid) methodology:

This demonstration project analyzed 21 split samples for a comparison between *E. coli* densities obtained from the IDEXX methodology and FC bacteria densities obtained from the MF methodology using m-FC media (with rosolic acid). A simple regression analysis of the log-normalized data is presented in Figure 4, below. The resultant Pearson correlation coefficient of 0.984 ($p < 0.0000$) indicates a very strong significant correlation between the two methodologies. The IDEXX methodology data is expressed MPN; while the MF methodology data is expressed in cfu/100mL. The formula for the regression line is: Log_{10} IDEXX *E. coli* = 0.0552704 + 1.19857*(Log_{10} MF fecal coliform).

Figure 4. Log_{10} IDEXX *E. coli* Densities vs. Log_{10} MF Fecal Coliform Densities



The *E. coli* densities obtained from the IDEXX methodology were consistently greater than the FC densities obtained from the MF (m-FC media with rosolic acid) methodology. The geometric mean % Error between the actual density data of the two methodologies was 206.0%. This should be interpreted as the IDEXX methodology enumerating significantly greater *E. coli* densities than the fecal coliform bacteria densities enumerated by the MF (m-FC media with rosolic acid) methodology.

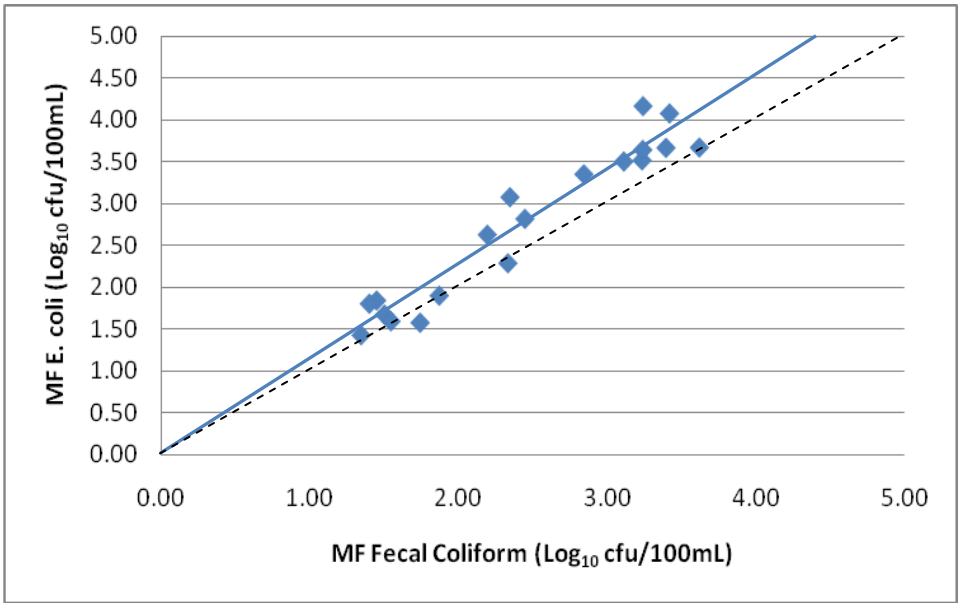
E. coli densities using the MF (m-ColiBlue24®) methodology vs FC densities using the MF (m-FC media with rosolic acid) methodology:

This demonstration project analyzed 19 split samples for a comparison between *E. coli* densities obtained from the MF (m-ColiBlue24® media) methodology and FC bacteria densities obtained from the MF (m-FC media with rosolic acid) methodology. A simple regression analysis of the log-normalized data is presented in Figure 5, below. The resultant Pearson correlation coefficient

of 0.963 ($p < 0.0000$) indicates a very strong significant correlation between the two methodologies. Both methodologies are expressed in cfu/100mL. The formula for the regression line is: Log_{10} MF m-ColiBlue24® *E. coli* = -0.00580088 + 1.13157*(Log_{10} MF fecal coliform).

The *E. coli* densities obtained from the MF (m-ColiBlue24® media) methodology were generally greater than the FC densities obtained from the MF (m-FC media with rosolic acid) methodology. The geometric mean % Error between the actual density data of the two methodologies was 43.3%. This should be interpreted as the MF (m-ColiBlue24® media) methodology enumerating significantly greater *E. coli* densities than the fecal coliform densities enumerated by the MF (m-FC media with rosolic acid) methodology.

Figure 5. Log_{10} MF *E. coli* Densities vs. Log_{10} MF Fecal Coliform Densities



Results of Sampling within the Lower Sub-basin JD 33.4

The RSBOJC performed sampling from January 2009 through February 2010 and consisted of a minimum of eight samples per sampling site. Of a total of 25 sites, 23 sampling sites were located within the lower sub-basin JD 33.4. The samples were then analyzed for *E. coli* by the IDEXX methodology in conformance with this demonstration project's QAPP. The raw data is presented in Appendix F.

Table 3, below, presents the geometric mean (geomean) and 90% value *E. coli* densities at each sampling site, as well as their pollution rankings and locations. A least mean square statistical analysis determined that sites 1, 6 and 7 had the lowest bacteria densities. Site 10 had the greatest bacteria densities. The second greatest and statistically equivalent bacteria densities were collected from sites 11, 14, 15, 16, 17, 18 and 19. The second lowest and statistically equivalent bacteria densities were collected from sites 21, 22, 23, 24 and 25.

The 90% value *E. coli* densities were calculated in specific compliance with second part of the dual criterion for fecal coliform bacteria established in the State of Washington Surface Water Quality Standards (Chapter 173-201A WAC). That part is written as "with not more than 10 percent of all samples obtained for calculating the geometric mean value exceeding 200 colonies/100 mL." The statistical "90th percentile" does not comply with the above wording. A 90% value density is, therefore, defined as: (1) the largest value in a dataset of less than 20 values; (2) the second largest value in a dataset of 20-29 values; (3) the third largest value in a dataset of 30-39 values; etc.

The lower sub-basin JD 33.4 contains eight smaller drainage basins, most of which were represented by multiple sampling sites. Table 4, below, presents the geometric mean and 90% value *E. coli* densities, as well as their pollution rankings and drainage descriptions.

Table 3. *E. coli* Geomean and 90% Values at Lower Sub-basin JD 33.4 Sampling Sites

Sampling Site	N	Geomean (MPN)	90% Value (MPN)	*E. coli* Pollution Ranking[4]	Location
1	8	3.4	1,413.6	11	1410 Rouse Rd.
2	8	303.3	3,255.0	7	Rouse Rd. across from Star Trailer Court #30
3	8	511.0	9,208.0	6	North Ave. & Rouse Rd., eastern-most manhole
4	8	1,318.2	6,131.0	5	Yakima Valley Highway & 9th St. (Carwash)
5	8	1,440.4	4,225.0	4	Nobel's parking lot, YVH between S. 6th St. & S. 9th St.
6	8	1.2	3.1	11	Warehouse Ave. between 6th St. & 7th St.; undeveloped
7	8	20.7	1,986.3	11	E. Blaine Ave. near alley
8	8	1,817.0	19,683.0	3	DR 3 past Edison in the alley by railroad
9	8	2,029.4	24,196.0	3	Decature & S. 9th St.
10	8	13,386.3	241,960.0	1	Decature Ave. between S. 9th St. & S. 8th St.
11	9	5,060.0	241,960.0	2	Decature Ave. between S. 8th St. & S. 7th St.
12	8	292.6	1,986.3	7	RSBOJC Site 25.15 at S. 1st St. & Zillah Ave.
13	8	264.1	2,419.6	7	E. Edison Ave. backyard (at Zillah Ave/Flower St)
14	8	6,747.7	19,863.0	2	Lincoln Ave. & S. 4th St.
15	8	5,945.6	12,997.0	2	JD 33.4 near Otis upstream of POTW discharge
16	8	5,840.5	9,804.0	2	JD 33.4 at S. 1st St. between S. Hill Rd. and E. Parkland
17	8	5,541.0	17,329.0	2	Midvale near Alexander Blvd.
18	8	2,982.9	9,208.0	2	Midvale near old rendering plant
19	8	3,663.5	19,863.0	2	RSBOJC Site 25.10 at Duffy Road
20	8	236.1	2,419.6	8	SCW at Duffy Rd.
21	9	113.7	2,613.0	9	South Hill Rd., Yakima Chief plant, in field by fence
22	8	94.0	866.4	9	South of I-82 in pasture with longhorn cattle
23	8	96.7	613.1	9	Emerald Rd. & Wells Rd.
24	8	172.6	2,419.6	9	DR 3 at Midvale, across from last hexed manhole
25	8	39.2	435.2	10	DR 3 at Port of Sunnyside lagoon

Table 4. *E. coli* Geomean and 90% Values at Lower Sub-basin JD 33.4 Drainages

Drainage	N	Sampling Sites	Drainage Description	Geomeean Mean	90% Value	*E. coli* Pollution Ranking[4]
1	64	1, 2, 3, 4, 5, 6, 7, 8	DR 3 - North	120.2	4,225.0	2
2	25	9, 10, 11	DR 3 – Decature	5,156.8	241,960.0	1
3	33	21, 22, 23, 24	DR 3 - West	115.5	2,419.6	2
4	8	25	DR 3 - East	39.2	435.2	2
5	8	12	JD 33.4 - West	292.6	1,986.3	2
6	32	13, 14, 15, 16	JD 33.4 - Middle	2,804.8	11,119.0	1
7	27	17, 18 19	JD 33.4 - South	3,926.8	17,329.0	1
8	8	20	SCW	236.1	2,419.6	2

[4] The pollution rankings are listed in numerical order, with the sites of greatest pollution being represented by the value 1. All sampling sites (Table 3) and drainages (Table 4) having the same numerical ranking are statistically equivalent by the "least mean squares" analysis.

A "least mean square" statistical analysis determined that least bacterial densities were found in drainage basin #1 (DR 3 – Upstream), which represents the uppermost areas of the lower Sub-basin JD 33.4, and in drainage basin #5 (DR 3 – East), which represents the eastern-most portion of the sub-basin. Drainage basins #2 (DR 3 – North) and #4 (DR 3 – West) all contained significantly greater bacterial densities, which would be expected as those areas each represent a significant increase in urban and agricultural activities, respectively.

Of special interest to this project is drainage basin #3 (DR 3 – Decature), since it the first drainage basin when moving downstream through the sub-basin that contains excessive bacterial densities. This drainage basin contains a large portion of downtown city of Sunnyside (Sunnyside) and contained the greatest geometric mean *E. coli* density. City of Sunnyside management was alerted by the RSBOJC to the results of bacterial sampling and the municipality subsequently repaired several sanitary sewage leaks along Decature Ave., which discharged directly into the stormwater sewer system. Such leaks were indeed found to be "discrete point sources" of high-strength bacterial discharges. Sunnyside management should consider an ongoing investigation of its sanitary sewer system for leaks and possible illicit connections.

The remaining areas of excessive *E. coli* bacterial densities (#6, #7 and #8) represent the drainage basins "JD 33.4 – Middle", "JD 33.4 – South" and "JD 33.4 – Downstream", respectively. The excessive bacterial densities in these last three drainage basins are suspected of primarily due to the result of carry-over from the upstream drainage. Once the city of Sunnyside has completed its leak/illicit connection survey of the municipal sewer system, then the excessive bacterial densities in these later drainage basins are expected to decrease substantially.

Conclusions

Project Goal #1:

The goal of "allowing extensive monitoring of bacterial pollution throughout a large agricultural watershed by a staff-limited and space-limited local agency" was successfully accomplished. During this demonstration project, the 2-person RSBOJC laboratory completed analysis of 220 additional samples with minimal impacts on their normal work load or work schedule. The total "hands-on" time required per sample was determined to be 15 minutes with the IDEXX methodology, as compared to 42 minutes with the MF methodologies (Appendix H). Although such per-sample times are greater than previously cited in the scientific literature, they are considered to be more exact as they include all "hands-on" procedures from set-up through clean-up. During this demonstration project, the use of the IDEXX methodology saved 27 minutes of "hands-on" time per sample, or approximately a total of 100 work hours.

Other scientific investigators have found the IDEXX methodology to be similarly rapid. Buckalew et al. (2006) stated that: "Colilert DST presents a laboratory protocol that is simpler to manage, quicker to process, and easier to quantify results than MF. These factors, plus the enhanced precision and versatility of Colilert DST over the span of this three-year study attests to its suitability for testing ambient surface waters." Yakub et al. (2002) stated that: "…for the most part, the DSM techniques provided a faster and less expensive alternative to traditional methods of bacterial enumeration." Finally, Macy et al. (2005) stated that: "Colilert is simpler to use, allows greater throughput, and requires less time to standardize than standard methods."

Project Goal #2:

The goal of "determining if the use of *E. coli* monitoring can be an acceptable surrogate for FC bacteria in watersheds" was successfully accomplished. Various researchers determined that the enumeration of *E. coli* by the IDEXX methodology is very strongly correlated to enumeration by the MF methodology for *E. coli*. Chang et al. (1989), Shadix and Rice (1991), Fricker et al. (1997), Ekner (1998), Hanko (2000), Jackson et al. (2002), Hargett and Goyn (2004), Hamilton et al. (2005), Dinkins (2006), Hörman and Häninnen (2006), Pitkänen et al. (2007), and Al-Turki and El-Ziney (2009) all discussed similar findings concerning Colilert®'s enumeration capability.

It should be noted that most of the above researchers also concluded that enzyme-specific media technologies (i.e. IDEXX) enumerated greater bacteria densities than MF technologies. In particular, Lifshitz and Joshi (1997) determined a regression coefficient of 0.98 and an average 45% greater enumeration with IDEXX methodology than that found with the MF methodology. The Ministry of the Environment, Canada (unpublished data) determined a regression coefficient of 0.95 and an average 32% greater enumeration than the MF methodology. During this project, the IDEXX methodology for *E. coli* produced a regression correlation of 0.984 and an average 41% greater enumeration than the MF (m-FC media with rosolic acid) methodology for fecal coliform. In a similar side-by-side comparison, Kloot et al. (2006) also found an average of 41% greater *E.coli* densities than fecal coliform densities.

Some researchers have suggested that the greater enumeration results obtained from the IDEXX methodology may be the result of a greater-than-average false-positive rate. However, Eccles et al. (2004) has determined a false-positive rate of only 1.59% and a false-negative rate of 3.81% with the IDEXX methodology. Other researchers have determined similarly low rates and hypothesized that the methodology actually is more "sensitive". Bonadonna et al (2007) stated that Colilert was a more appropriate procedure for the identification of *E. coli* than the traditional MF methodologies (i.e. m-ColiBlue®), as more than 35% of the species are now known to be lactose negative.

The increased "sensitivity" of the enzyme-based methodologies (i.e. IDEXX) is also suspected of being indirectly due to the high number of false negatives associated with the MF methodologies. Schauer et al. (2005) compared ten USEPA approved enzyme-based *E. coli* analysis methodologies at the University of Wisconsin on spiked water samples. They determined that the IDEXX methodology had a 0% failure rate to detect the presence or absence of the bacteria; whereas, the MF (m-ColiBlue24® media) exhibited a 23% failure rate. Garcia-Armisen et al. (2005) suggested that underestimation of fecal coliform in the MF methodology may be due to a high proportion of injured and of viable but non-culturable (VBNC) bacterial cells, which represent substantial false negatives (Wutor et al., 2007).

The concept of injured and VBNC bacteria cells not being enumerated by MF methodologies has been discussed by researchers since the 1980's. McFeters et al. (1982) concluded: "Most of the media commonly used in water analysis recovered less than 30% of injured cells." and that: "Injury is an important factor in underestimating numbers of waterborne indicator bacteria which may lead to inaccurate public health assessments." Rompré at al. (2002) determined that it is not possible to recover injured or VBNC bacteria colonies while using the standard agar media in common use for MF methodologies.

From all of the above results and discussion, this demonstration project has determined that the IDEXX methodology for *E. coli* analysis is an acceptable surrogate for analysis of FC bacteria by the MF methodology. This determination is similar to that made by the Slovenia Equivalence Verification Study of July 2007, which concluded that the IDEXX methodology is "…easier, more sensitive and reliable, and quicker to use than the reference (MF) methodology" and that it is "an acceptable and indeed more sensitive method for recovery of *E. coli* from water".

Project Goal #3:

The goal of "accelerating the development of required bacterial TMDLs and, thereby, accelerate the improvement of an agricultural watershed's water quality" was successfully accomplished. This demonstration project has shown that the IDEXX methodology successfully allowed a small rural water quality laboratory to locate potential several "discrete point sources" of high-strength bacterial pollution within a combined agricultural/urban sub-basin. The sampling located several municipal sanitary sewer leaks discharging into JD 33.4, which were quickly repaired by the City of Sunnyside. The repair of the sewer leaks most assuredly accelerated water quality improvement in the SCW watershed. However, only future long-term effectiveness monitoring in the SCW will be able to ultimately determine the actual degree of such improvement.

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APPENDIX A

Colilert®/Quanti-Tray 2000 Accuracy Quality Assurance Tests

MicroBiologics® Epower Microorganisms
E. coli known density: 7,800 cfu/pellet
Epower lot number: #483711
Epower expiration date: 2011-03
Date analysis was performed: 6/11/09

One pellet was dissolved in 100mL of pH 7.2 buffer solution and divided evenly among 10 sample replicates. Although the original Measurement Quality Objective (MQO) was listed in this project's QAPP as "<25% RPD", Ecology revisited the issue and suggested using a more appropriate MQO of 25% to 175% Recovery. The replicates were analyzed in the RSBOJC water quality laboratory using the IDEXX methodology. Only one of the 10 samples exceeded the revised % Recovery MQO indicated above.

Percent Recovery

Replicate #	Known Density (cfu/100mL)	Analysis Result (MPN)	%Recovery
1	7,800	12,997	166.6
2	7,800	14,136	181.2
3	7,800	9,208	118.0
4	7,800	10,462	134.1
5	7,800	11,199	143.6
6	7,800	10,462	134.1
7	7,800	9,804	125.7
8	7,800	10,462	134.1
9	7,800	11,199	143.6
10	7,800	12,033	154.8
Mean			143.6

APPENDIX B

Colilert®/Quanti-Tray 2000 Precision Quality Assurance Tests

Each sample was field-split and then analyzed in the RSBOJC water quality laboratory. The replicates were analyzed using the IDEXX methodology. The Measurement Quality Objective for precision was originally set in the QAPP as "<25% RPD of log-normalized data", but was subsequently re-established by Ecology's EAP Program as "<40% RPD" based on the actual data. Only two of the 20 samples exceeded the revised MQO for precision.

Relative Percent Difference on Actual Data

Sampling Site #	Date of Sample	Replicate A Analysis Result (MPN)	Replicate B Analysis Result (MPN)	RPD
12	2/10/2009	40.8	35.0	15.3
17	3/19/2009	17,329.0	15,531.0	10.9
20	3/23/2009	15.3	14.5	5.4
4	3/25/2009	1,267.0	1,119.0	12.4
22	4/16/2009	133.4	121.1	9.7
11	5/14/2009	2,755.0	1,789.0	42.5
9	6/1/2009	3,968.0	4,352.0	9.2
1	6/10/2009	4.1	4.1	0
18	6/29/2009	9,208.0	11,199.0	19.5
14	8/5/2009	6,876.0	5,172.0	28.3
19	9/29/2009	5,475.0	3,225.0	51.7
22	10/13/2009	112.6	137.4	19.8
13	11/2/2009	112.6	88.6	23.9
22	11/4/2009	69.5	69.1	0.6
23	11/4/2009	63.8	52.8	18.9
15	12/1/2009	7,701.0	7,701.0	0
12	12/9/2009	42.0	37.3	11.9
5	12/10/2009	1,533.1	1,119.9	31.1
24	12/16/2009	313.0	387.3	21.2
25	12/16/2009	5.1	4.1	21.7
Mean				17.7

APPENDIX C

Comparison of IDEXX and MF (ColiBlue24®) Methodologies for *E. coli*

The IDEXX methodology is composed of the Colilert®/Quanti-Tray 2000 technologies and is enumerated in MPN. The MF methodology utilizes the ColiBlue24® technology and is enumerated in cfu/100mL. IDEXX Laboratories, Inc. suggests that their MPN values are numerically equivalent to cfu/100mL values. % Error = ((Idexx Value – MF Value)/MF Value) x 100. The % Error results were not normally distributed, which required the use of a geomean rather than an arithmetic mean.

Sampling Site #	Date of Sample	Idexx Value (MPN)	MF Value (cfu/100mL)	% Error
12	2/10/2009	40.8	47.4	-13.9
4	3/25/2009	1,267.0	660.0	92.0
14	9/17/2009	5,172.0	400.0	1,193.0
15	9/17/2009	8,164.0	1,200.0	580.3
2	10/8/2009	2,247.0	1,200.0	87.3
17	10/8/2009	4,884.0	3,200.0	52.6
24	10/8/2009	727.0	427.0	70.3
22	10/13/2009	112.6	80.0	40.8
15	10/19/2009	11,199.0	4,700.0	138.3
16	10/19/2009	8,664.0	3,300.0	162.5
19	10/19/2009	3,654.0	2,250.0	62.4
20	10/19/2009	648.8	195.0	232.7
25	10/19/2009	71.2	39.5	80.3
13	11/2/2009	112.6	38.0	196.3
18	11/2/2009	9,208.0	4,400.0	109.3
22	11/4/2009	69.5	70.0	-0.7
23	11/4/2009	63.8	27.0	136.3
8	11/12/2009	19,683.0	14,667.0	34.2
9	11/16/2009	17,329.0	12,000.0	44.4
15	12/1/2009	7,701.0	4,666.0	65.0
12	12/9/2009	42.0	64.0	-34.4
Geomean				41.4

APPENDIX D

Comparison of IDEXX Methodology for *E. coli* vs MF Methodology for FC

The IDEXX methodology is composed of the Colilert®/Quanti-Tray 2000 technologies and is enumerated in MPN. The MF methodology utilizes the m-FC (with rosalic acid) technology and is enumerated in cfu/100mL. % Error = ((Idexx Value – MF Value)/MF Value) x 100. The % Error results were not normally distributed, which required the use of a geomean rather than an arithmetic mean.

Sampling Site #	Date of Sample	Idexx Value (MPN)	MF Value (cfu/100mL)	% Error
12	2/10/2009	40.8	31.6	29.1
4	3/25/2009	1,267.0	280.0	352.5
1	6/10/2009	4.1	3.0	36.7
18	6/29/2009	9,208.0	2,000.0	360.4
2	10/8/2009	2,247.0	222.0	912.2
17	10/8/2009	4,884.0	1,300.0	275.7
24	10/8/2009	727.0	156.0	366.0
22	10/13/2009	112.6	74.0	52.2
15	10/19/2009	11,199.0	4,200.0	166.6
16	10/19/2009	8,664.0	1,725.0	402.3
19	10/19/2009	3,654.0	700.0	422.0
20	10/19/2009	648.8	215.0	201.8
25	10/19/2009	71.2	35.0	103.4
13	11/2/2009	112.6	55.0	104.7
18	11/2/2009	9,208.0	1,744.0	428.0
22	11/4/2009	69.5	28.0	148.2
23	11/4/2009	63.8	22.0	190.0
8	11/12/2009	19,683.0	1,752.0	1,023.5
9	11/16/2009	17,329.0	2,640.0	556.4
15	12/1/2009	7,701.0	2,500.0	208.0
12	12/9/2009	42.0	25.0	68.0
Geomean				206.0

APPENDIX E

Comparison of MF Methodology for *E. coli* vs MF Methodology for FC

The EC-MF methodology for *E. coli* utilizes the ColiBlue24® technology and is enumerated in cfu/100mL. The FC-MF methodology for FC bacteria utilizes the m-FC (with rosalic acid) technology and is enumerated in cfu/100mL. % Error = ((Idexx Value – MF Value)/MF Value) x 100. The % Error results were not normally distributed, which required the use of a geomean rather than an arithmetic mean.

Sampling Site #	Date of Sample	EC-MF Value (cfu/100mL)	FC-MF Value (cfu/100mL)	% Error
12	2/10/2009	47.4	31.6	50.0
4	3/25/2009	660.0	280.0	135.7
2	10/8/2009	1,200.0	222.0	440.5
17	10/8/2009	3,200.0	1,300.0	146.2
24	10/8/2009	427.0	156.0	173.7
22	10/13/2009	80.0	74.0	8.1
15	10/19/2009	4,700.0	4,200.0	11.9
16	10/19/2009	3,300.0	1,725.0	91.3
19	10/19/2009	2,250.0	700.0	221.4
20	10/19/2009	195.0	215.0	-9.3
25	10/19/2009	39.5	35.0	12.9
13	11/2/2009	38.0	55.0	-30.9
18	11/2/2009	4,400.0	1,744.0	152.3
22	11/4/2009	70.0	28.0	150.0
23	11/4/2009	27.0	22.0	22.7
8	11/12/2009	14,667.0	1,752.0	737.2
9	11/16/2009	12,000.0	2,640.0	354.5
15	12/1/2009	4,666.0	2,500.0	86.6
12	12/9/2009	64.0	25.0	156.0
Geomean				43.3

APPENDIX F

E. coli Sampling Data throughout the Lower Sub-basin JD 33.4

Date of Sampling	Sampling Site #	*E. coli* (MPN)
02/02/09	1	1.0
03/19/09	1	1.0
05/20/09	1	1.0
06/10/09	1	4.1
07/28/09	1	1,413.6
10/08/09	1	3.1
11/02/09	1	1.0
12/09/09	1	1.0
02/02/09	2	6.2
03/19/09	2	90.9
05/20/09	2	12.1
06/10/09	2	344.8
07/28/09	2	2,419.6
10/08/09	2	2,247.0
11/02/09	2	1,723.0
12/09/09	2	3,255.0
02/02/09	3	90.9
03/19/09	3	285.1
05/20/09	3	23.8
06/10/09	3	613.1
07/28/09	3	9,208.0
09/21/09	3	1,725.0
10/28/09	3	1,413.6
12/10/09	3	547.5
02/02/09	4	172.5
03/25/09	4	1,267.0
04/16/09	4	2,419.6
06/10/09	4	1,553.1
07/28/09	4	6,131.0
09/21/09	4	906.0
10/28/09	4	1,413.6
12/10/09	4	1,413.6
02/02/09	5	178.2
03/25/09	5	4,225.0
04/16/09	5	2,419.6
06/10/09	5	3,448.0
07/28/09	5	1,986.3
09/21/09	5	988.0
10/28/09	5	980.4
12/10/09	5	1,533.1
02/02/09	6	1.0
03/17/09	6	1.0
04/16/09	6	1.0
07/08/09	6	1.0

08/05/09	6	3.1
09/21/09	6	1.0
11/12/09	6	1.0
12/10/09	6	1.0
01/29/09	7	3.0
03/17/09	7	1.0
04/30/09	7	4.0
06/01/09	7	1,986.3
07/22/09	7	93.3
09/21/09	7	73.3
10/28/09	7	209.8
12/10/09	7	1.0
01/29/09	8	2,419.6
03/25/09	8	243.0
04/30/09	8	435.0
06/01/09	8	14,136.0
07/21/09	8	1,723.0
09/21/09	8	6,488.0
11/12/09	8	19,863.0
12/09/09	8	148.0
01/29/09	9	2,419.6
03/25/09	9	813.0
05/14/09	9	44.3
06/01/09	9	1,986.3
07/21/09	9	24,196.0
09/29/09	9	12,997.0
11/16/09	9	17,329.0
12/09/09	9	305.0
01/29/09	10	2,419.6
03/25/09	10	24,196.0
05/14/09	10	19,863.0
06/01/09	10	6,867.0
07/21/09	10	24,196.0
09/29/09	10	241,960.0
10/28/09	10	30,760.0
12/14/09	10	717.0
01/29/09	11	2,419.6
03/25/09	11	2,382.0
05/14/09	11	1,553.1
06/01/09	11	7,270.0
09/29/09	11	241,960.0
12/14/09	11	3,654.0
12/21/09	11	6,131.0
01/04/09	11	2,613.0
02/11/10	11	2,359.0
02/10/09	12	40.8
03/30/09	12	56.3
04/30/09	12	461.1
06/01/09	12	1,119.9
07/22/09	12	1,986.3
09/17/09	12	350.0
11/02/09	12	1,553.1
12/09/09	12	42.0

03/11/09	13	20.4
04/09/09	13	2,419.6
05/07/09	13	203.0
06/29/09	13	1,850.0
07/21/09	13	1,162.0
09/29/09	13	279.0
11/02/09	13	112.6
12/09/09	13	35.0
02/10/09	14	2,419.6
03/23/09	14	19,863.0
04/30/09	14	4,884.0
07/08/09	14	7,270.0
08/05/09	14	6,867.0
09/17/09	14	5,172.0
11/02/09	14	9,208.0
12/01/09	14	7,701.0
02/10/09	15	1,986.3
03/23/09	15	12,997.0
04/30/09	15	4,884.0
07/01/09	15	2,419.6
08/05/09	15	7,270.0
09/17/09	15	8,164.0
10/19/09	15	11,199.0
12/01/09	15	7,701.0
02/10/09	16	2,419.6
03/23/09	16	9,804.0
04/30/09	16	3,873.0
07/01/09	16	6,131.0
08/05/09	16	5,794.0
09/17/09	16	4,884.0
10/19/09	16	8,664.0
12/01/09	16	9,804.0
02/10/09	17	2,419.6
03/19/09	17	17,329.0
04/30/09	17	6,488.0
06/29/09	17	6,131.0
07/22/09	17	4,352.0
10/08/09	17	4,884.0
11/02/09	17	7,701.0
12/16/09	17	3,255.0
03/11/09	18	2,419.6
04/09/09	18	3,255.0
05/07/09	18	292.0
06/29/09	18	9,208.0
07/28/09	18	2,419.6
10/13/09	18	1,725.0
11/02/09	18	9,208.0
12/17/09	18	7,701.0
02/18/09	19	1,413.6
03/23/09	19	1,413.6
04/16/09	19	2,723.0
07/08/09	19	3,448.0
08/10/09	19	19,863.0

09/29/09	19	5,475.0
10/19/09	19	3,654.0
12/16/09	19	4,352.0
02/18/09	20	2,419.6
03/23/09	20	15.3
04/16/09	20	228.2
07/08/09	20	275.5
08/10/09	20	260.3
09/29/09	20	198.9
10/19/09	20	648.8
12/16/09	20	123.6
03/30/09	21	2.0
04/16/09	21	1.0
12/14/09	21	1.0
12/21/09	21	260.3
01/04/10	21	2,419.6
01/14/10	21	1,203.3
01/21/10	21	980.4
01/27/10	21	816.4
02/11/10	21	2,613.0
02/18/09	22	2.0
03/30/09	22	186.0
04/16/09	22	133.4
07/08/09	22	866.4
08/10/09	22	547.5
10/13/09	22	112.6
11/04/09	22	69.5
12/14/09	22	33.2
02/18/09	23	2.0
03/19/09	23	579.4
05/14/09	23	209.8
06/29/09	23	613.1
07/22/09	23	325.5
10/13/09	23	98.5
11/04/09	23	63.8
12/14/09	23	25.0
02/18/09	24	2.0
03/30/09	24	48.7
05/07/09	24	2,419.6
07/15/09	24	214.2
08/05/09	24	305.0
10/08/09	24	727.0
11/12/09	24	224.7
12/16/09	24	313.0
02/18/09	25	1.0
03/30/09	25	44.1
05/07/09	25	98.8
07/15/09	25	435.2
08/05/09	25	68.3
09/17/09	25	117.8
10/19/09	25	71.2
12/16/09	25	5.1

APPENDIX G

Quality Assurance Project Plan

Quality Assurance Project Plan

Alternative Bacteria Source Identification using Colilert®/Quanti-Tray 2000 Test Method in Irrigated Agricultural Watersheds

Environmental Assessment Program
Olympia, Washington 98504-7710

October 2008

Quality Assurance Project Plan

Alternative Bacteria Source Identification using Colilert®/Quanti-Tray 2000 Test Method in Irrigated Agricultural Watersheds

October 2008

Approvals

Gregory Bubin, Ecology WQP, Project Manager, CRO	Date 11/12/2008
Ryan Anderson, Ecology WQP, TMDL Projects Coordinator, CRO	Date 11/12/08
Jon Merz, Ecology WQP, Non-point Unit Supervisor, CRO	Date 11/12/08
Robert Barwin, Ecology, WQP, Section Manager, CRO	Date 11/12/08
Elaine Brouillard, RSBOJC, Water Quality Specialist	Date 10-27-2008
James W. Trull, District Manager, Sunnyside Valley Irrigation District	Date 11/03/08
Tom Monroe, District Manager, Roza Irrigation District	Date 11/5/08
Mike Herold, Ecology WQP, Quality Assurance Officer, HQ	Date 11/17/08

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Tables and Figures

Abstract

The Washington State Department of Ecology (Ecology) received grant funds for Innovative TMDL Development Projects from the United States Environmental Protection Agency (EPA), Region 10. The demonstration project will evaluate whether *E. coli* can be effectively used by a small rural laboratory as a substitute for fecal coliform (FC) bacteria monitoring for the identification of bacteria sources in an irrigated agricultural watershed.

The Sulphur Creek Wasteway (SCW) watershed is divided into seven sub-basins based on its various tributaries (Figure 1). The Roza-Sunnyside Board of Joint Control (RSBOJC) has conducted extensive FC bacteria monitoring throughout all of the sub-basins of the SCW. All but one of the sub-basins had significantly greater FC bacteria densities during the *irrigation season* "critical condition". Only the lower sub-basin JD 33.4 showed a *year-round* "critical condition", which suggests the predominant bacteria sources in that sub-basins are "point sources" rather than "non-point sources".

Several additional water samples will need to be analyzed throughout the lower sub-basin JD 33.4 in order to locate more precisely the point source discharges of bacterial pollution. However, the RSBOJC, like various rural agencies, has limited staff and laboratory footprint/equipment for analyzing water samples. The demonstration project will specifically utilize an alternative methodology, Colilert®/Quanti-Tray 2000, for the analysis of *E. coli* as a substitute for the labor-intensive membrane filtration analysis of FC bacteria.

A positive outcome from the demonstration project will provide an efficient alternative method for bacteria source identification in primarily irrigated agricultural watersheds. The use of Colilert®/Quanti-Tray 2000, if successful, is expected to allow future bacterial TMDLs in those types of watersheds to be completed on an accelerated timeline.

Background

General Description

The Sulphur Creek Wasteway (SCW) watershed is located in Yakima County within Water Resource Inventory Area (WRIA) 37 with its center located at Latitude: 46.27861 and Longitude: -120.00056 (Figure 1). The watershed occupies lands surrounding the city of Sunnyside, which is located on the east side of the Cascade Mountain range alongside Interstate 82, approximately 35 miles southeast of the city of Yakima and 45 miles northwest of the Tri-cities area (Richland/Kennewick/Pasco).

The 96,000-acre (150 square miles) watershed lies in the Yakima River valley and is bounded by the Horse Heaven Hills anticline to the south and the Rattlesnake Hills anticline to the north. The soil is deep, well-drained, fertile silt loam (Zuroske, 2004). The natural vegetation of the SCW watershed is categorized as shrub-steppe, consisting of various sagebrushes and bunchgrasses. However, when the land is cleared and irrigation water is applied, an unlimited variety of crops can be grown in the SCW watershed.

The climate of the area is generally characterized as mild and dry. Summer air temperatures range from 85 to over 100 degrees Fahrenheit. Winters are generally cool with air temperatures often falling below freezing from November through January, sometimes reaching 20 degrees below zero Fahrenheit. Annual snowfall is light and averages 10 to 15 inches. The SCW watershed receives 6 to 8 inches of annual precipitation, with the principle growing season (June, July and August) receiving less than one inch of measurable precipitation.

The SCW is a man-made drainage canal (7.5 miles in length) that ultimately collects all of the irrigation return flows, municipal stormwater, and some point source municipal and industrial discharges that occur within the watershed. It was constructed in 1908-1910 and serves as the Sunnyside Division's principal man-made drainage canal. The drainage canal system is owned by the United States Bureau of Reclamation and is part of that agency's Yakima Project.

Land-uses

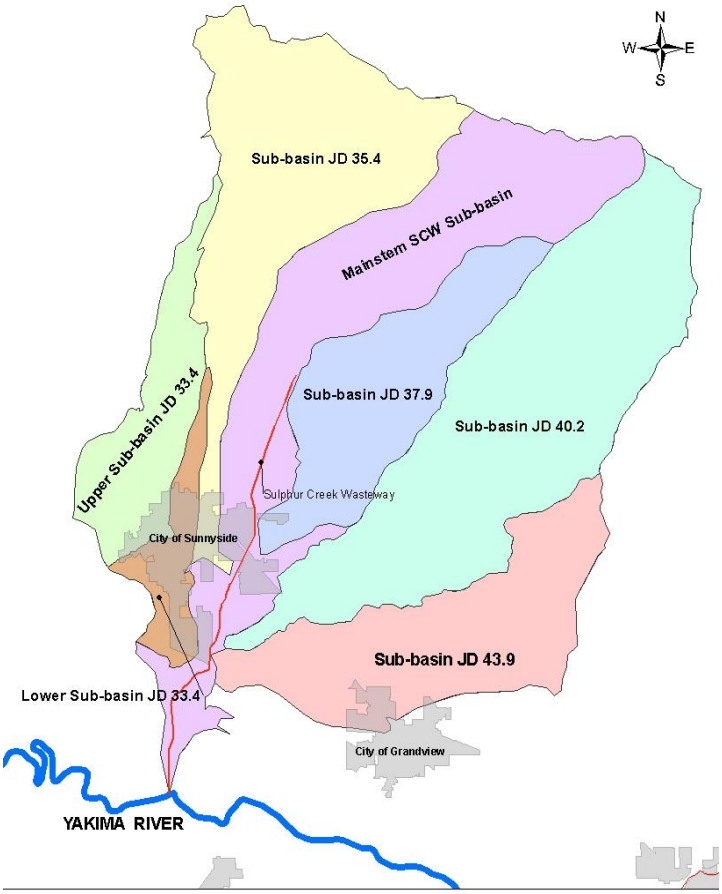
In addition to approximately 1,500 acres of irrigated agricultural lands, the lower sub-basin JD 33.4 includes two dairies, part of one municipality, several "hobby farms" and rural residences. The drainage from the sub-basin includes runoff from irrigation agriculture, stormwater flows from the central business district of the City of Sunnyside, effluent discharges from the City's POTW, and discharges from the entire Port of Sunnyside industrial property.

Bacterial Problem

Analysis of water quality data from 1968 through 1985 by the United States Geological Survey (USGS) found that the SCW watershed FC bacteria densities were "among the largest observed throughout the Yakima River Basin" (Embry, 1992). Morace et al. (1999) determined that agricultural practices caused increased fecal-indicator bacteria in streams that receive irrigation return flows throughout the lower Yakima River basin. That same report and another by Fuhrer et al. (2004) suggested that the excessive FCB densities in the SCW were related to the watershed's high density of livestock. Ecology water quality monitoring near the mouth of the SCW found FC bacteria geometric means of 1,237 cfu/100mL (2 samples) and 1,437 cfu/100mL (9 samples) during 1993 and 1994, respectively.

The RSBOJC's Ecology-certified laboratory has also collected a vast amount of FC bacteria density data from the SCW and its various tributaries since 1997. Figure 1 shows the seven sub-basins of the composing the SCW watershed. The greatest bacterial pollution in the watershed is found in the lower sub-basin JD 33.4. These high densities are found year-round. All of the other sub-basins have significantly lower bacteria densities during the non-irrigation period (November 1 – March 31).

Figure 1. Sulphur Creek Watershed Sub-basins



The demonstration project is specifically directed at the lower sub-basin JD 33.4, because its irrigation season (April 1 – October 31) FC bacteria densities continue to exist throughout the non-irrigation season. The year-round occurrence of high bacteria densities suggests that the predominant sources of bacterial pollution in the lower sub-basin JD 33.4 are "point sources" rather than "non-point sources" as suspected in all of the other SCW sub-basins.

Table 1 details the annual geometric mean (geomean) FC bacteria densities found during the irrigation season at each of the seven historical RSBOJC sub-basins within the SCW watershed, from 1999 through 2005. The greatest overall sub-basin irrigation season geomean (1999-2005 data combined) FC density (1,213 cfu/100 mL) occurred in the lower sub-basin JD 33.4.

Table 2 details the annual geometric mean (geomean) FC bacteria densities found during the non-irrigation season at each of the seven historical RSBOJC sub-basins within the SCW watershed, from 1999 through 2005. The greatest overall sub-basin non-irrigation season geomean (1999-2005 data combined) FC density (1,663 cfu/100 mL) occurred in the lower sub-basin JD 33.4.

Table 1. Irrigation Season Geomean FC Bacteria Densities in SCW Sub-basins, 1999-2005

Sub-basin # → / Year of Sampling ↓	Lower JD 33.4	Upper JD 33.4	JD 43.9	JD 40.2	JD 35.4	JD 37.9	Main stem SCW
1999	3,726	1,092	1,130	1,436	508	359	800
2000	815	715	801	1,181	251	353	457
2001	507	344	610	860	134	221	361
2002	1,540	-	301	580	172	196	410
2003	775	243	301	592	160	249	285
2004	1,003	319	451	633	250	136	364
2005	1,327	309	333	791	218	299	369
Geomean	1,213	448	534	875	244	262	498

Table 2. Non-irrigation Season Geomean FC Bacteria Densities in SCW Sub-basins, 1999-2005

Sub-basin # → / Year of Sampling ↓	Lower JD 33.4	Upper JD 33.4	JD 43.9	JD 40.2	JD 35.4	JD 37.9	Main stem SCW
1999	833	370	314	272	200	120	278
2000	1,937	251	164	129	100	72	383
2001	3,047	340	256	193	135	43	333
2002	1,117	-	209	268	520	247	355
2003	1,169	61	186	121	62	28	329
2004	1,350	112	135	190	433	83	283
2005	3,600	70	170	140	136	62	632
Geomean	1,663	139	196	179	175	75	400

Project Components

General Description

The demonstration project has the goal of showing whether numerous water quality samples can be efficiently analyzed for *E. coli* using the Colilert®/Quanti-Tray 2000 methodology by a small rural laboratory, without the concurrent need to increase laboratory staffing or footprint/floor space.

Numerous additional water quality samples are required to locate the bacteria point sources in the lower sub-basin JD 33.4. However, the RSBOJC's water quality laboratory (2 persons and limited floor space) would be quickly overwhelmed by the influx of numerous samples for the membrane filtration FC bacteria analysis. The demonstration project will utilize an alternative methodology that has already been approved by the EPA for conducting multiple *E. coli* analyses more rapidly than the laboratory's present time-consuming membrane filtration methodology.

This QAPP provides a review of the quality assurance (QA) measures associated with the Colilert®/Quanti-Tray 2000 methodology proposed for the demonstration project. The demonstration project requires the collection of eight water samples at each of twenty-five different sampling sites (total of 200 samples), all of which are located throughout the lower sub-basin JD 33.4. Thirty of those samples are required to be either split in the field or in the laboratory for QA purposes. All data collection and analyses shall be required to follow the applicable QA requirements found in this QAPP.

E. coli as a Substitute Indicator of Bacterial Pollution

The various RSBOJC historical paired *E. coli* and FC bacteria densities collected within the SCW watershed are numerically nearly equivalent. Use of the substitute bacteria indicator of *E. coli* is logical as such species also typically compose 95-98% of the FC bacteria in water samples collected throughout the State (Hicks, 2002).

Colilert®/Quanti-Tray 2000 Methodologies

The Colilert® methodology is used for the simultaneous detection and confirmation of *E. coli* in fresh and marine waters. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When *E.coli* metabolize the nutrient indicator, MUG, the sample fluoresces. EPA officially approved the Colilert® method for testing ambient waters (July 21, 2003 Federal Register). Same as Standard Methods (SM) enzyme substrate test: SM 9223(B).

Although membrane filtration and Colilert®/Quanti-Tray 2000 methodologies both need 24 hours for bacteria enumeration, the Colilert® methodology is more labor efficient: start to finish of 6 minutes hands-on time per sample. In addition, an incubator should be purchased that will easily accommodate several Quanti-Trays at the same time so that laboratory space efficiency will be maximized.

Each water sample is collected using a Colilert 100-ml pre-sterilized vial. Each vial then receives the contents of one MUG substrate packet. The vial is quickly resealed, shaken until contents are dissolved and then poured into a Quanti-Tray 2000 tray. The Quanti-Trays are then sealed and placed into an incubator at 35°C for 24 hours. An ultraviolet light is used to make the actual counts of the fluorescent yellow *E. coli* colonies.

Each Quanti-Tray contains 97 wells of two different sizes to achieve bacterial counts of 1 to 2,419 cfu/100mL, with a 95% confidence limit. For higher densities, it will be necessary to make dilutions of the collected water samples.

Objective and Desired Outcome

The demonstration project's objective is to prove whether, or not, the Colilert®/Quanti-Tray 2000 methodology will allow for the rapid analysis of numerous water samples without a concurrent need for increasing laboratory personnel or square footage. The project's desired outcome is to promote the accelerated development of future TMDLs in irrigated agricultural watersheds, especially those containing a large component of AFOs. A secondary desired outcome is to gain laboratory accreditation for the Colilert®/Quanti-Tray 2000 methodology, at the RSBOJC laboratory. Accreditation is required for Ecology to use test results in water quality-based actions.

Required Tasks

The principal tasks required by the demonstration project of the RSBOJC are as follows:

- Purchase all equipment needed for the demonstration project
- Determine and identify all sampling sites
- Collect water quality samples and conduct bacteria analyses
- Review all data for quality and compile qualified data
- Deliver all data to Ecology for preparation of final report
- Disseminate copies of final report to interested parties and put onto RSBOJC website

The principal tasks required by the demonstration project of Ecology are as follows:

- Obtain signatures for QAPP and Interagency Agreement
- Statistically analyze data
- Prepare final report for demonstration project
- Deliver final report to RSBOJC

Project Schedule

The sampling portion of the demonstration project is expected to commence by January 1, 2009 and continue through December 31 of that same year. It may be necessary, however, to adjust the timelines presented in Table 3, below, if special circumstances arise.

Table 3. Project Schedule

Schedule of Project Activities and Reports	
Schedule of Activities:	
Select fifteen additional sampling sites	by December 31, 2008
Purchase Colilert®/Quanti-Tray 2000	by November 30, 2008
Purchase laptop computer and software	by January 31, 2009
Purchase all other necessary consumables	by December 31, 2008
Begin collection and analysis of water quality samples	by January 15, 2009
Schedule of Reports:	
Draft Report due for RSBOJC peer review	by March 15, 2010
Draft Report due for Ecology peer review	by April 15, 2010
Final Report due for Ecology approval	by June 15, 2010
Final Report due to RSBOJC for dissemination	by June 30, 2010

Organization and Responsibilities

Table 4 presents the organization and responsibilities of the principal Ecology and RSBOJC staff that are associated with this QAPP and the demonstration project.

Table 4. Organization and Responsibilities of Staff

Stakeholder Name	Agency	Title	Primary Responsibilities
Gregory Bohn	Ecology	WQP, CRO, Demonstration Project Manager	Responsible for development of the final QAPP and the final report for the demonstration project.
Elaine Brouillard	RSBOJC	Local Agency Lead	Responsible for collecting/analyzing water samples and delivery of data to the Project Manager.
Ryan Anderson	Ecology	WQP, CRO, TMDL Projects Coordinator	Responsible for approval of the final QAPP and the final project report.
Jon Merz	Ecology	WQP, CRO, Unit Supervisor	Responsible for approval of the final QAPP and the final project report.
Robert Barwin	Ecology	WQP, CRO, Section Manager	Responsible for approval of the final QAPP and the final project report.
James W. Trull	Sunnyside Valley Irrigation District	District Manager	Responsible for compliance with the requirements of the Interagency Agreement.
Tom Monroe	Roza Irrigation District	District Manager	Responsible for compliance with the requirements of the Interagency Agreement.
Mike Herold	Ecology	WQP, HQ, QA Officer	Responsible for approval of the final QAPP.

Project Budget

The entire cost of the demonstration project is estimated to be $30,000, the majority of which represents the Colilert®/Quanti-Tray 2000 equipment and labor costs. The costs associated with the FCB membrane filtration analyses are only for consumables as the RSBOJC laboratory has already purchased the required equipment. The RSBOJC will be given an Ecology grant for the total amount above and will be required to sign an interagency agreement to provide for the collection and analysis of water quality samples as outlined in this QAPP.

Budget Category				**Budget Category Total**
Personnel	Hourly Wage	Hours	Sub-totals	
Project Manager	$35.00	20	$700*	
Water Quality Specialist	$25.00	220	$5,500	
Water Quality Tech	$15.00	220	$3,300	**$9,500**
Fringe Benefits (16% of salaries)				
Includes: retirement, health care, annual and sick leave, life insurance				**$1,520**
Transportation ($0.70 per mile)				
Includes: depreciation, gas, oil, and maintenance				**$230**
Equipment				
Colilert®/Quanti-Tray-2000				**$9,525**
Supplies			Sub-totals	
Office supplies: paper, pens, toners for printers and copiers			$750	
Consumables for Colilert® methodology			$900	
Consumables for FCB membrane filtration methodology			$570	
Laptop PC for data collection in the field			$1,500	
Software for laptop and in-house laboratory PC			$825	**$4,545**
Other				
Print and mail project information to agencies and local community				**$1,700**
Total Direct Costs				**$27,020**
Indirect Costs (17% of base)				
Base = total direct cost of $27,020 less capital equipment costs				**$2,974**
Total Project Costs				**$29,994**

* Amount will be retained by the RSBOJC for project incidentals, as Project Manager will be paid by Ecology.

Data Quality Objectives

Data quality objectives are statements of the precision, bias, and lower reporting limits necessary in order to generate data that address project objectives. Sampling, laboratory analysis, and data evaluation steps have several sources of error that should be addressed by data quality objectives. The primary determinants for data quality are accuracy, precision and bias.

The RSBOJC laboratory will ultimately obtain accreditation for the Colilert®/Quanti-Tray 2000 methodology, if the demonstration project proves successful. It is acknowledged that such accreditation would be very beneficial if obtained prior to the bacterial analyses required by this QAPP, so that the collected data could be placed into Ecology water quality program's data base.

Accuracy

Accuracy measures how close laboratory results are to a true or expected value. Accuracy is usually measured by analyzing a sample "spiked" with a known concentration. For purposes of the project, no "spikes" will be used, but rather the true value is assumed to be that obtained from FC bacteria membrane filtration analysis. Ten random samples (5% of total samples) will be split in the laboratory and analyzed by the Colilert®/Quanti-Tray 2000 methodology for *E. coli*, as well as the membrane filtration methodology for both *E. coli* and FC bacteria. Because *E. coli* is assumed to make up 95-98% of FC bacteria agricultural watersheds, accuracy will be estimated by the relative percent difference (RPD) calculated from comparative analysis of the laboratory split samples. Only log-transformed data will be used to assess accuracy.

Precision

Precision, expressed as RPD and derived from analysis of field sample splits, is a measure of the reproducibility of a result while subject to random error. Random error can occur during sample handling, preservation, and storage, as well as during the analytical process. Twenty random samples (10% of total samples) will be split in the field and analyzed by the Colilert®/Quanti-Tray 2000 methodology for *E. coli*. Precision will be estimated by the RPD calculated from comparative analysis of the field split samples. Only log-transformed data will be used to assess precision.

Bias

Bias is a statistical measurement of the difference due to systematic errors between the result for a parameter and the true value. Potential sources of systematic errors include sample collection, physical and chemical instability of samples, interference effects, instrument calibration, and contamination. There are no specific numerical levels of bias defined for bacterial analyses. Bias will be assumed to have been minimized by adherence with established quality control protocols outlined in this QAPP and in the RSBOJC laboratory's own standard operating procedures. Table 5 presents the Measurement Quality Objectives (MQOs) for this QAPP.

Table 5. MQOs for Project

Analysis	Method	Accuracy	Precision	Bias	Required Reporting Limits
FCB	SM 9222-D	n/a	<25% RPD*	n/a	1 cfu/100mL
E. coli	SM 9223-B	<25% RPD[a]	<25% RPD[b]	n/a	1 MPN/100mL

[a] RPD using log-transformed data compared to laboratory split samples measured by the FC bacteria membrane filtration methodology.
[b] RPD using log-transformed data comparing field split samples.

Sampling Process Design

The twenty-five sampling sites will be established for the demonstration project, all located along JD 33.4 in the lower sub-basin, because that area shows a "year-round" bacterial critical condition. Thus, the demonstration project suspects that the principal bacteria sources in the sub-basin are "point sources". Ten easily-accessible monitoring sites (Appendix A) have been pre-selected by Ecology and RSBOJC in order to divide the entire length of above sub-basin into easily-accessible segments. The RSBOJC Water Quality Specialist is required to select fifteen additional sampling sites prior to commencement of sampling in order to further divide the lower sub-basin JD 33.4.

All twenty-five sampling sites will each be sampled a minimum of eight times for a total of 200 samples to be collected throughout the duration of the project. The samples will be collected on random days and at random times in order to prevent any potential bias by time-sensitive discharges. No more than one sample per site shall be collected in any single month. The RSBOJC may collect and analyze more than 200 samples, but the additional samples should be distributed equally throughout the various months of the project duration. No sampling shall be collected within 24 hours after any precipitation (storm) event.

Ten samples of the required minimum of 200 are required to be split in the field and analyzed for precision QA (See Table 5, above). Ten samples of the required minimum of 200 are required to be split in the laboratory and analyzed for accuracy QA (See Table 5, above). No more than one field split sample shall be obtained from any single site.

Sampling Procedures

Safety procedures for field sampling, developed by the RSBOJC, shall be followed at all times. Field sampling shall be postponed any time personnel determine that driving conditions, site access, or sampling conditions are unsafe. Field sampling shall resume as soon as possible, but only after safe conditions have returned. All samples will be collected as grab samples of water from as near the middle of the stream flow, as possible.

Collected samples shall be immediately placed into pre-cleaned and sterilized glass or plastic containers (bottles or "whirlpaks"). Containers shall be appropriately labeled (sample ID, date, time and sampling site), and subsequently transferred to a cooler and placed in crushed or cube ice (0°C to 4°C) for short-term storage. All cooled samples shall be delivered to the RSBOJC laboratory within 6 hours after sample collection. At the laboratory, the cooled samples shall be analyzed for *E. coli*, or FC bacteria when required, within 24 hours after collection.

Table 6 outlines the details of the required field sampling procedures.

Table 6. Field Sampling Procedures

Parameter	Container Size	Container Type	Preservation	Delivery Time to Lab	# of Sampling Sites	# of Samples per Site	# of Samples to be Split in Field for QC
Bacteria	250 or 500 mL	Glass or Plastic	Cool to 4°C	within 6 hours	25	8	10*

* To be collected at the discretion of the sampling personnel from different sites.

Laboratory Procedures

Laboratory analysis of FC bacteria shall be performed in accordance with RSBOJC standard operating procedures, which utilizes the EPA-approved method: SM9222-D (membrane filter method). Laboratory analysis of *E. coli* shall be performed in accordance with the manufacture's manual, which utilizes the EPA-approved method: SM9223-B (Colilert®/Quanti-Tray/2000).

Sampling staff will continually communicate with the RSBOJC laboratory to ensure that all sampling and laboratory analyses will proceed on schedule with a minimum of confusion. The sampling staff will follow normal RSBOJC procedures for sample notification and scheduling. It is expected that with adequate communication, sample quantities should not overwhelm the capacity of the small two-person RSBOJC laboratory. Use of the Colilert®/Quanti-Tray 2000 equipment should allow the RSBOJC laboratory to keep up with the numerous bacterial samples that would otherwise not be possible.

Table 7 outlines the details of the required laboratory procedures.

Table 7. Laboratory Procedures

Analyte	Analytical Method	Expected Range of Results	Maximum Holding Time	# of Samples to be Split in the Laboratory for QC
FCB	SM 9222-D	50 - 5,000 cfu/100mL	24 hours	10*
E. coli	SM 9223-B	50 - 5,000 MPN/100mL	24 hours	N/A

* To be collected at the discretion of the laboratory personnel. All split samples will be analyzed for both *E. coli* and FC bacteria.

Calibration and Quality Control

All equipment utilized for laboratory analyses will be checked and calibrated, if required, as per the manufacturer's directions. All laboratory chemicals, reagents and other materials will be properly stored and checked for expiration dates. All expired materials will be appropriately discarded and at no time will be used for bacterial analyses.

Total variation for field sampling and analytical variation will be estimated by analysis of field split samples and comparing such variation to the precision MQO listed in Table 5, above. Bacteria samples tend to have a high RPD compared to other water quality analyses. Total variation for field sampling and laboratory analysis of bacterial samples will be estimated by analyzing twenty field split samples, all collected from different sites. Precision up to a maximum RPD of 25% of log-transformed data will be considered acceptable for field split samples.

Ten of the collected samples will be split in the laboratory for measurement of *E. coli* by both the Colilert®/Quanti-Tray 2000 and membrane filtration methodologies, as well as for FC bacteria by the membrane filtration methodology. Accuracy up to a maximum RPD of 25% of log-transformed data will be considered acceptable for laboratory split samples.

Employing consistent and standard procedures will ensure that individual samples are representative of the water conditions at the times and places they are collected. The time of day when sampling sites are visited will be randomized, with no sampling site being visited at the same time of day. The ambient conditions will be documented at time of sampling so that later correlations may be made of the data. All samples will be collected in pre-cleaned plastic sample containers, placed in crushed or cube ice (0°C to 4°C), and delivered to the RSBOJC laboratory within 6 hours of collection.

Data Management Procedures

Field notes will either be entered directly into the laptop computer or entered into a notebook with waterproof pen, as soon as possible after the collection of each sample. Such notes shall include the time of day, date of collection, sample ID, and sampling site location or ID #. As laboratory analysis data are collected, they will be compiled and entered into an electronic spreadsheet for analysis. After all samples have been collected, the geometric mean and 90% values of *E. coli* (and of FC bacteria when necessary) will be calculated for each sampling site and compared to the State's water quality criteria for FC bacteria.

Non-detect values shall be treated as 0 cfu/100 mL, as the lower detection limit of the Colilert®/Quanti-Tray 2000 methodology is 1 cfu/100 mL. In addition, the RPD values for both accuracy and precision will be calculated and compared to the maximum value of 25%. All of the above values represent important aspects of the demonstration project. All field and laboratory data shall be completed and delivered to the Ecology Project Manager by December 31, 2009.

Data Verification and Validation

Data Verification

Data verification involves examining the data for errors, omissions, and compliance with quality control (QC) acceptance criteria. The local agency lead (RSBOJC Water Quality Specialist) will be responsible for performing the following functions:

- Reviewing and reporting QC checks on instrument performance such as initial and continuing calibrations.
- Reviewing and reporting the results of field and laboratory QC sampling/analyses.
- Explaining flags or qualifiers were assigned, where necessary, to sample results.
- Reviewing and assessing the RSBOJC laboratory's performance in meeting the conditions and requirements set forth in this QAPP.
- Reporting the above information to the Ecology Project Manager.

After measurement results have been recorded and prior to sending such data to the Ecology Project Manager, such person will verify that:

- Data are consistent, correct, and complete, with no errors or omissions.
- Established criteria for QC results were met.
- Data specified in Sampling Process Design were obtained.
- Methods and protocols specified in the QAPP were followed.

Field results will also be verified by RSBOJC staff before leaving the sampling site after each sample collection event. Detailed field notes will be kept to meet the requirements for documentation of field measurements. RSBOJC staff is responsible for checking and ensuring that field data entries are complete and error free.

Data Validation

Data validation is the next step following verification. Data validation involves a detailed examination of the data using professional judgment to determine whether the MQOs for precision, bias, and accuracy have been met, as well as whether the data is complete. The Ecology Project Manager will assess both accuracy and precision of the data, since bias is not possible to numerically assess. RPD analyses of field split samples (precision) and laboratory split samples (accuracy) will be conducted. Acceptable accuracy and precision performance is defined as an RPD of <25% (log-transformed data).

The Ecology Project Manager will examine the completeness of the data by its compliance with procedures outlined in this QAPP; whereas, the RSBOJC Water Quality Specialist will examine completeness of the data by its compliance with procedures outlined in the RSBOJC laboratory's standard operating procedures. Completeness will be assessed by examining: number of samples collected compared to sampling plan; correctness of actual sampling sites compared to sampling plan; number of samples collected by and received in acceptable condition at the RSBOJC laboratory; the laboratory's ability to produce usable results for each sample; and sample results accepted by the Water Quality Specialist.

Data Quality Assessment

After the data have been verified and validated, the following steps will be conducted to assess the data quality:

- Review the data quality objectives and the sampling design.
- Conduct a preliminary data review.
- Apply appropriate statistical tests to evaluate the bacterial data.
- Verify the assumptions of the statistical test.
- Draw conclusions from the bacterial data.

Any result that fall outside the accuracy and precision MQO of <25% RPD (log-transformed data) will be evaluated individually to see if any laboratory or sampling errors were made during the project. The Project Manager will use best professional judgment in the decision as to whether these values will be eliminated from the data set. If no such errors have been determined to have been made, then all such data must be utilized in the project's calculations.

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Hicks, M. (2002) *Setting Standards for the Bacteriological Quality of Washington's Surface Water – Draft Discussion Paper and Literature Summary*. Washington State Department of Ecology. Publication 00-10-072. Revised December 2002. 109 pp.

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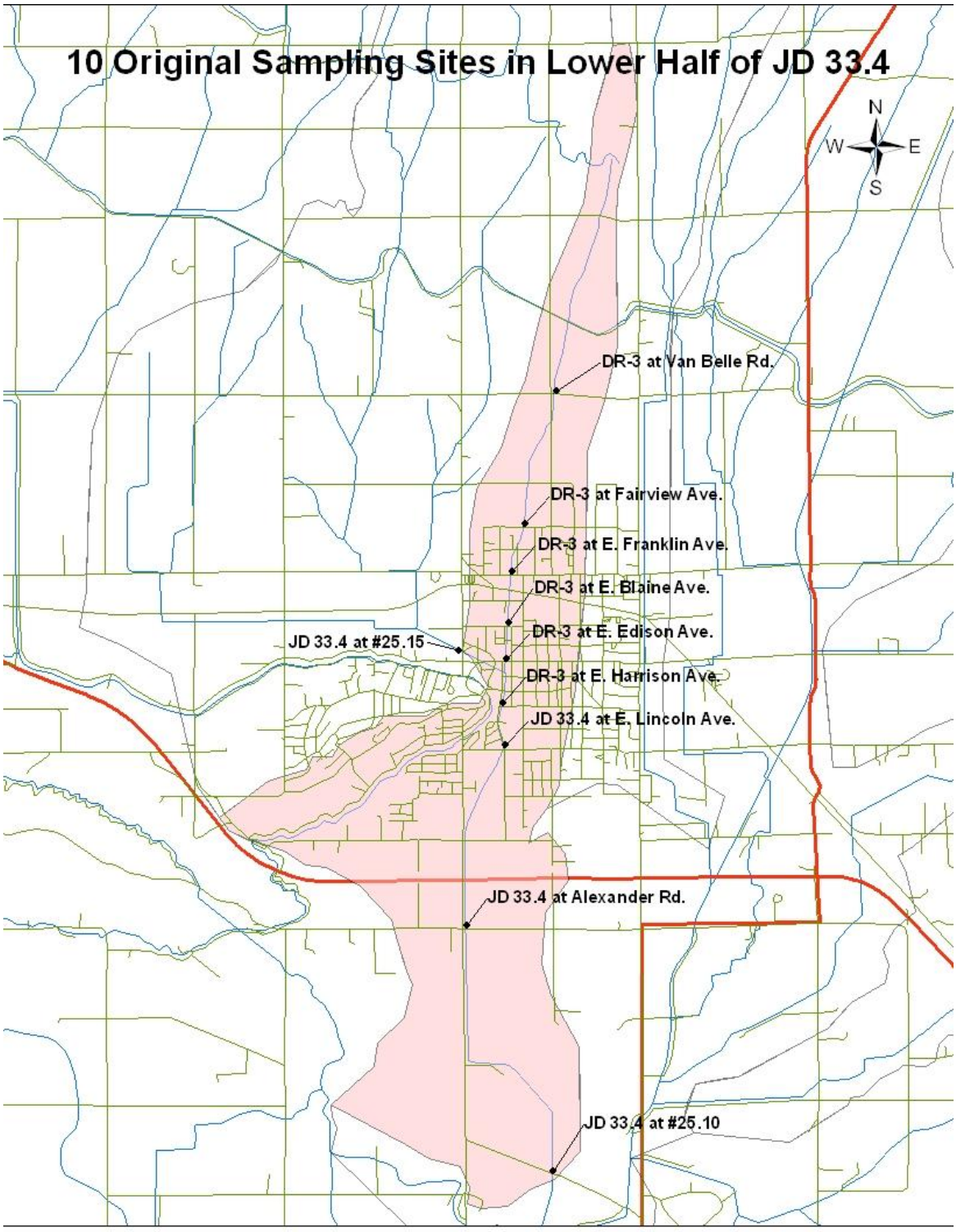
Appendix A. Original 10 sampling sites in lower sub-basin JD 33.4



APPENDIX H

Average Time Difference between IDEXX and MF Methodologies

Laboratory Proceedure	IDEXX Methodology per Quanti-Tray[1] (minutes)	MF Methodology per Plate[2] (minutes)
Set-up	2	5
Run	4	2
Breakdown	2	5
Read & calculate	1	3
Wash	1	4
Autoclave[3]	50	50
Total per unit	60	72

[1] Times are typical for one IDEXX Quanti-Tray that is diluted 1:10 and includes the mixing and resting periods for the enzymatic reaction. However, two Quanti-Trays were frequently used per each water quality sample. Two Quanti-Trays in tandem would take approximately **65** minutes to acquire an enumerated value.

[2] Times are typical for one MF plate. However, three plates were run per each water quality sample, along with two "before and after" plates for QA/QC. Each entire 5-plate process would take approximately **92** minutes to acquire an enumerated value.

[3] Times for autoclaving are listed but are included in the calculation of "hands-on" times.