Michigan Department of Environmental Quality

Water Bureau

Total Maximum Daily Load for *E. coli* for the Detroit River

Wayne, Oakland, and Washtenaw Counties, Michigan

August 25, 2008

1.0 INTRODUCTION

Section 303(d) of the federal Clean Water Act and the United States Environmental Protection Agency's (USEPA's) Water Quality Planning and Management Regulations (Title 40 of the Code of Federal Regulations, Part 130) require states to develop Total Maximum Daily Loads (TMDLs) for water bodies that are not meeting water quality standards (WQS). The TMDL process establishes the allowable loadings of pollutants for a water body based on the relationship between pollution sources and in-stream water quality conditions. TMDLs provide states a basis for determining the pollutant reductions necessary from both point and non-point sources to restore and maintain the quality of their water resources. The purpose of this TMDL is to identify the allowable levels of *E. coli* that will result in the attainment of the applicable WQS in the Detroit River, located in Wayne County, Michigan.

The Detroit River is a unique water body in that it is a connecting channel between two of the Great Lakes (Huron and Erie) and is an international water body. Its watershed consists of United States (U.S.) and Canadian lands and its water surface is divided by the international boundary. As an important ecological and navigational waterway, the management of the Detroit River is accomplished with guidance from the Great Lakes Water Quality Agreement (GLWQA) of 1972, a Bi-national Agreement that is coordinated by the International Joint Commission.

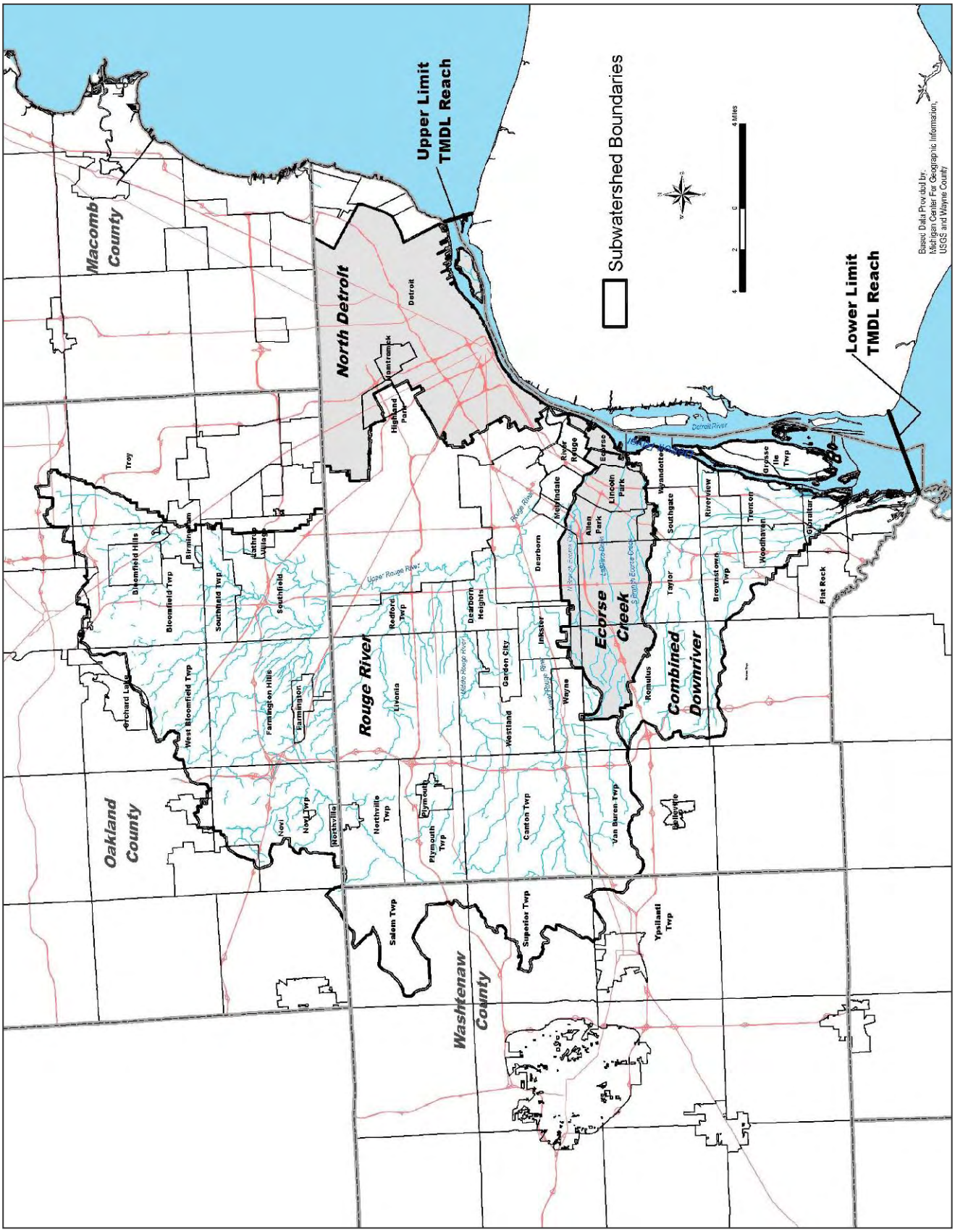
This U.S. *E. coli* TMDL does not apply to sources of *E. coli* within the Canadian portion of the Detroit River watershed, although those sources contribute to the measured water quality impairments. Consequently, it is necessary to proportion the Detroit River flow to calculate a loading capacity that accounts for, and allocates, U.S. loads only. This TMDL acknowledges that achieving water quality standards will require continued cooperation between the U.S. and Canada under the GLWQA in addition to successful implementation of this TMDL by U.S. agencies and stakeholders.

The U.S. side of the Detroit River watershed includes portions of Oakland, Wayne, and Washtenaw counties in southeast Michigan. Portions of Ontario, Canada, also drain to the Detroit River. There are four subwatersheds in the U.S. portion of the watershed: Rouge River, Ecorse River, North Detroit, and Combined Downriver (See Figure 1). The Rouge River is the largest subwatershed comprising 69% of the Detroit River watershed in the U.S. The Ecorse River subwatershed makes up another 6% of the watershed. The MDEQ developed, and the USEPA approved, an *E. coli* TMDL for the Rouge River in 2007 (MDEQ 2007). The Ecorse River *E. coli* TMDL is currently under development. Together, these two TMDLs address sources of *E. coli* in 75% of the U.S. Detroit River watershed.

The Detroit River *E. coli* TMDL has been developed consistent with the allocations provided in the draft and final *E. coli* TMDLs for the Ecorse and Rouge rivers, respectively, and does not attempt to further address *E. coli* sources in those watersheds. Successful implementation of the Ecorse River and Rouge River *E. coli* TMDLs will result in attainment of the total and partial body contact designated use at those rivers' confluences with the Detroit River. Attainment of the *E. coli* WQS for the Detroit River *E. coli* TMDL relies on concurrent implementation of the *E. coli* TMDLs for the Rouge and Ecorse rivers.

The remaining drainage areas in the Detroit River watershed are comprised of the Combined Downriver and North Detroit subwatersheds. Both subwatersheds are located entirely within Wayne County. The Combined Downriver area is drained by the Frank and Poet Drain, Brownstown Creek and Blakely Drain.

Figure 1. Detroit River Watershed (U.S. portion only)



The Combined Downriver area comprises 13% of the U.S. Detroit River watershed. Approximately 31% of the Combined Downriver subwatershed drains directly to the Detroit River via overland flow and sewers, including a combined sewer system in Wyandotte and a portion of Southgate. Grosse Ile, an island in the Detroit River, is part of the Combined Downriver subwatershed.

The North Detroit subwatershed comprises 12% of the U.S. Detroit River watershed. The City of Detroit represents most of the land area in this subwatershed. The City of Detroit is almost entirely drained by a combined sewer system, which includes 46 outfalls along the Detroit River shoreline. The remainder is drained by separate storm sewers, which discharge directly to the Detroit River. The communities of Hamtramck, Highland Park, River Rouge, and Ecorse are fully or partially located in the North Detroit subwatershed.

2.0 PROBLEM STATEMENT

The TMDL reach for the Detroit River appears on the 2008 Section 303(d) list as (LeSage and Smith, 2008):

Water Body Name: Detroit River **AUID:** 040900040001-01

Impaired Designated Use: Partial Body and Total Body Contact Recreation

Cause: *Escherichia coli* **Size:** 23.73 miles

Location: Lake Erie u/s to Lake St. Clair. Excludes 2 miles of Critical Assessment Zones of Detroit River drinking water intakes.

TMDL Year: 2008

The location will be modified in the 2010 Integrated Report to more explicitly define the impaired reach. The upper and lower limits of the modified reach will be as follows: the upstream limit at Lake St. Clair begins on the U.S. shoreline at Latitude 42.358, Longitude -82.928 and ends on the Canadian shoreline at Latitude 42.340, Longitude -82.921. The downstream limit at Lake Erie begins on the U.S. shoreline at Latitude 42.032, Longitude -83.189 and ends on the Canadian shoreline at Latitude 42.051, Longitude -83.113. The upstream limit at the U.S. shoreline divides the North Detroit subwatershed and Lake St. Clair watershed, while the downstream limit at the U.S. shoreline divides the Combined Downriver subwatershed and Huron River watershed (See Figure 1).

The Detroit River was placed on the Section 303(d) list due to the presence of combined sewer overflows and *E. coli* levels exceeding Michigan's water quality standard (WQS) for total and partial body contact recreation. Monitoring data collected by the Michigan Department of Environmental Quality (MDEQ) in 2007 documented exceedences of the WQS for *E. coli* during the total body contact recreational season of May 1 through October 31. Elevated *E. coli* levels have been observed through the years in sampling conducted on this water body by the City of Detroit.

3.0 NUMERIC TARGET

The impaired designated uses addressed by this TMDL are total and partial body contact recreation. The designated use rule (R 323.1100 of the Part 4 rules, WQS, promulgated under

Part 31, Water Resources Protection, of the Natural Resources and Environmental Protection Act, 1994 PA 451, as amended) states that this water body is to be protected for total body contact recreation from May 1 to October 31 and partial body contact recreation year-round. The target levels for this designated use are the ambient *E. coli* standards established in Rule 62 of the WQS as follows:

R 323.1062 Microorganisms.
Rule 62. (1) All waters of the state protected for total body contact recreation shall not contain more than 130 E. coli per 100 milliliters, as a 30-day geometric mean. Compliance shall be based on the geometric mean of all individual samples taken during 5 or more sampling events representatively spread over a 30-day period. Each sampling event shall consist of 3 or more samples taken at 2 representative locations within a defined sampling area. At no time shall the waters of the state protected for total body contact recreation contain more than a maximum of 300 E. coli per 100 milliliters. Compliance shall be based on the geometric mean of 3 or more samples taken during the same sampling event at representative locations within a defined sampling area.
(2) All surface waters of the state protected for partial body contact recreation shall not contain more than a maximum of 1,000 E. coli per 100 milliliters. Compliance shall be based on the geometric mean of 3 or more samples, taken during the same sampling event, at representative locations within a defined sampling area.

The target for sanitary wastewater discharges is:

Rule 62. (3) Discharges containing treated or untreated human sewage shall not contain more than 200 fecal coliform bacteria per 100 milliliters, based on the geometric mean of all of 5 or more samples taken over a 30-day period, nor more than 400 fecal coliform bacteria per 100 milliliters, based on the geometric mean of all of 3 or more samples taken during any period of discharge not to exceed 7 days. Other indicators of adequate disinfection may be utilized where approved by the department.

Sanitary wastewater discharges are considered in compliance with the WQS of 130 *E. coli* per 100 milliliters (mL) if their National Pollutant Discharge Elimination System (NPDES) permit limit of 200 fecal coliform per 100 mL as a monthly average is met. This is assumed because *E. coli* are a subset of fecal coliform (APHA, 1995). Fecal coliform concentrations are substantially higher than *E. coli* concentrations alone when the wastewater of concern is sewage (Whitman, 2001). Therefore, it can reasonably be assumed that there are fewer than 130 *E. coli* per 100 mL in the effluent when the point source discharge is meeting its limit of 200 fecal coliform per 100 mL.

The target for this TMDL is 300 *E. coli* per 100 mL expressed as a daily maximum load and concentration from May 1 to October 31 (i.e., daily target) and 130 *E. coli* per 100 mL as a 30-day geometric mean, expressed as a concentration (i.e., monthly target). An additional target is the partial body contact standard of 1,000 *E. coli* per 100 mL as a daily maximum concentration year round. Achievement of the total body contact daily maximum target is expected to result in attainment of the partial body contact standard.

3.1 LINKAGE ANALYSIS

Determining the link between the *E. coli* concentrations in the Detroit River and the potential sources is necessary to develop the TMDL. TMDLs must be established at a level necessary to

attain and maintain the applicable WQS. Because the waste load allocations (WLA) - the loading associated with point source discharges - provided herein are based on the daily target, a linkage analysis is needed to demonstrate these allocations also assure attainment of the monthly target.

The USEPA's development of ambient water quality criteria for bacteria, as contained in the "Ambient Water Quality Criteria for Bacteria – 1986" (USEPA, 1986), defines the statistical relationship between the daily maximum and 30-day geometric mean criteria values. The assumption used to develop the 30-day geometric mean of 126 cfu per 100 mL (rounded to 130 cfu per 100 mL as the Michigan criterion) is a log-normal distribution using a log standard deviation of 0.4. Using this assumption and a comparable recurrence interval (e.g., 30 days), a daily maximum projected from the 30-day geometric mean would be 713 cfu per 100 mL. Conversely, the Michigan daily maximum criterion of 300 cfu per 100 mL is comparable to a 30-day geometric mean of approximately 55 cfu per 100 mL. This relationship provides the basis for demonstrating that attaining the daily target in the TMDL will also achieve the monthly target. A further conservative assumption is the log standard deviation of 0.4. The log standard deviation observed in most riverine systems is generally at least 0.3, and often quite larger (Cleland, 2006). The greater the variability, the more protective the daily maximum is relative to the monthly target.

Michigan regulates discharges containing treated or untreated human waste (i.e., sanitary wastewater) using fecal coliform. Sanitary wastewater discharges are required to meet 200 fecal coliform per 100 mL as a monthly average and 400 fecal coliform per 100 mL as a maximum. The MDEQ believes the sanitary wastewater discharges are in compliance with the daily and monthly targets and the allocations associated with the daily target, if their NPDES permit limits for fecal coliform are met. The *E. coli* criteria contained in the 1986 document were derived to approximate the degree of protection (i.e., 8 illnesses per 1,000 swimmers) provided by the fecal coliform indicator level of 200 cfu per 100 mL recommended by the USEPA prior to the adoption of the 1986 criteria. All wastewater treatment plants (WWTPs) provide year-round disinfection, providing another level of confidence that these sources will not contribute to *E. coli* WQS exceedences in the receiving waters.

4.0 DATA DISCUSSION

This portion of the document is divided into two main sections: hydrologic data and *E. coli* data. The hydrology section describes the available flow data for the Detroit River and how flow estimates were calculated. The *E. coli* section presents and summarizes the ambient *E. coli* data that were collected during 2007. Further information on the *E. coli* data collection effort can be found in the document *Detroit River and Ecorse River E. coli Monitoring to Support TMDL Development Final Report*, which is included as Attachment A.

4.1 Hydrology Data

The Detroit River is a major water resource shared by the United States and Canada. The United States and Canada measured flow in the Detroit River independently until 1953 when the Coordinating Committee on Great Lakes Basic Hydraulic and Hydrologic Data (Coordinating Committee) was established to unify flow measurement and reporting between the two nations. The Coordinating Committee serves in an advisory capacity to the agencies of the United States and Canada who are charged with the responsibility for collecting and compiling the Great Lakes hydraulic and hydrologic data. In the United States, the Detroit District of the Army Corps of Engineers (USACE) is the lead agency for this data collection effort.

To date, empirical stage-fall-discharge relationships have been determined to be the best predictors of flow in the Detroit River (Koschik, 2008; Quinn, 1979). The USACE and the National Oceanic and Atmospheric Administration (NOAA) maintain four gaging stations that record Detroit River water levels from Lake St. Clair to Lake Erie. Ontario maintains two gaging stations in addition to the U.S. stations. The USACE estimates a single flow each day for the entire Detroit River using the stage-fall-discharge regression equations and stage data collected at the four gaging stations (Quinn, 1979). Incoming flows from tributaries such as the Rouge and Ecorse rivers are considered to be negligible by the USACE (Koschik, 2008). The average flow of the Detroit River is approximately 187,000 ft^3/s (Holtschlag and Koschik, 2001).

Estimated daily mean flow data were obtained from the USACE, Detroit District (Koschik, 2007) for the period 1977 to 2007, providing 30 years of flow record for developing flow duration curves. The 30-year record represents a range of prevailing climatic conditions and long-term cyclical water levels in the Great Lakes, and therefore is deemed an adequate record of flow for use in this TMDL. Because the Detroit River is influenced to a much greater degree by lake levels than precipitation, the flow duration curve reflects long-term Great Lakes water levels more so than annual precipitation variability. Attachment B contains plots of the flow time series and duration curve.

Because this TMDL only applies to U.S waters, it is necessary to portion flow between U.S. and Canadian waters for the purpose of calculating the loading capacity and load allocations (see Section 7). The USGS and USACE collected Acoustic Doppler Current Profiler (ADCP) data in 2002 (Holtschlag and Koschik, 2003). The ADCP data provide depth and velocity data across numerous cross-sections in the Detroit River between Lake St. Clair and Lake Erie, including cross-sections near six of the *E. coli* monitoring transects (DR-1, DR-2, DR-3, DR-4, DR-6, and DR-8) discussed in Section 4.2.1. The ADCP data in closest proximity to the six transects were obtained from the USACE, Detroit District (Koschik, 2007) and analyzed with WinRiver 10.06 software, which calculates flow using the ADCP data. Using ADCP data from locations nearest to the U.S. shoreline and international boundary, the flow for the U.S. portion of the Detroit River was determined at the six transects. The flow proportion at those six transects ranged from 45.5% at transect DR-8 to 68.4% at DR-1 with a mean value of 52.3%. This mean value was used to determine the U.S portion of the Detroit River flow.

4.2 *E. coli* Data

4.2.1 Study Design

Nine monitoring transects were established on the Detroit River. Transects were roughly evenly spaced over the course of the River, with one each placed at the most upstream and downstream ends of the River. There were multiple sampling sites associated with each transect. The number of sampling sites was based on the width of the river. Three to nine sampling sites were roughly evenly spaced across the river in both U.S. and Canadian waters. The left and right most sites were generally within 50 feet of the shorelines. Sampling site “A” always corresponds to the left most sample nearest to the U.S. mainland. The sampling transects and their corresponding sampling sites are described in Table 1 and depicted in Figures 2a and 2b.

All monitoring sites were sampled for *E. coli* for 23 weeks, from May 1, 2007, through October 2, 2007. A single sample was collected at each transect sampling location for a total of 1,300 samples over the 23 week study period.

Table 1. Detroit River Transect Descriptions (listed upstream to downstream)

Transect	Sampling Sites	Transect Description
DR0	A – E	Outlet of Lake St. Clair and upstream end of Windmill Pointe Park and upstream of Peche Island
DR1	A – E	Upstream of Belle Isle and downstream of Little River
DR2	A – C	Scott Middle Ground near the Detroit Boat Club, north side of Belle Isle
DR2	D – F	Fleming Channel, south side of Belle Isle
DR3	A – E	Downstream of the GM Renaissance Center
DR4	A – E	At Fort Wayne
DR5	A – E	Downstream of the Rouge River cut-off channel and Zug Island
DR6	A – E	Downstream of the Ecorse River
DR6	F – H	North end of Fighting Island to the downstream of Turkey Creek
DR7	A – C	Trenton Channel near Elizabeth Park
DR7	D – I	Grosse Ile to downstream of the Canard River
DR8	A	Near Brownstown Creek outlet
DR8	B – I	Upstream of Lake Erie and downstream of Celeron Island to the Canadian mainland

For the purpose of calculating geometric mean values, single-sample results for each of the Detroit River transects were divided into two or three groups based on the location of various land masses within the River and by the location of the international boundary. Separate geometric means were determined for U.S. and Canadian waters (Table 2). A minimum of three *E. coli* single samples were used for the geometric mean calculation per the Michigan WQSs. For transects that only had two samples (DR0 and DR5), a third value was obtained by interpolating between the nearest two samples. For instance, at transect DR0 only two samples, A and B, were collected in U.S. waters (See Figure 2a). A third value was estimated by interpolating between sampling site B and C. These three values were used to calculate the geometric mean for the U.S. portion of the transect.

At the transect along Grosse Ile, three daily geometric means were calculated: one for the Trenton Channel on the west side of Grosse Ile, one for the east side of Grosse Ile in U.S. waters and one for east side of Grosse Ile in Canadian waters. Upstream of Lake Erie along transect DR8, sample A was handled separately because it was located away from the rest of the samples, near the outlet of Brownstown Creek.

The 2007 sampling budget allowed for the collection and analysis of four bacterial source tracking (BST) samples to determine if human *E. coli* sources were present in the river. The four sample locations were selected based on the frequency and magnitude of any elevated *E. coli* results found during the first 3 ½ months of the 2007 sampling. At that time, the sample locations in the U.S. portion of the Detroit River had sufficiently low *E. coli* readings that BST sampling was not warranted. As a result, the BST sampling occurred at locations found in Canadian waters only. Note that the U.S. side of the river had WQS exceedences after the BST samples were taken. This information would have likely changed the BST sampling locations, but was not available when the sites were selected. A discussion of the results of this effort can be found in Attachment A.

Figure 2a. Upper Detroit River Monitoring Locations

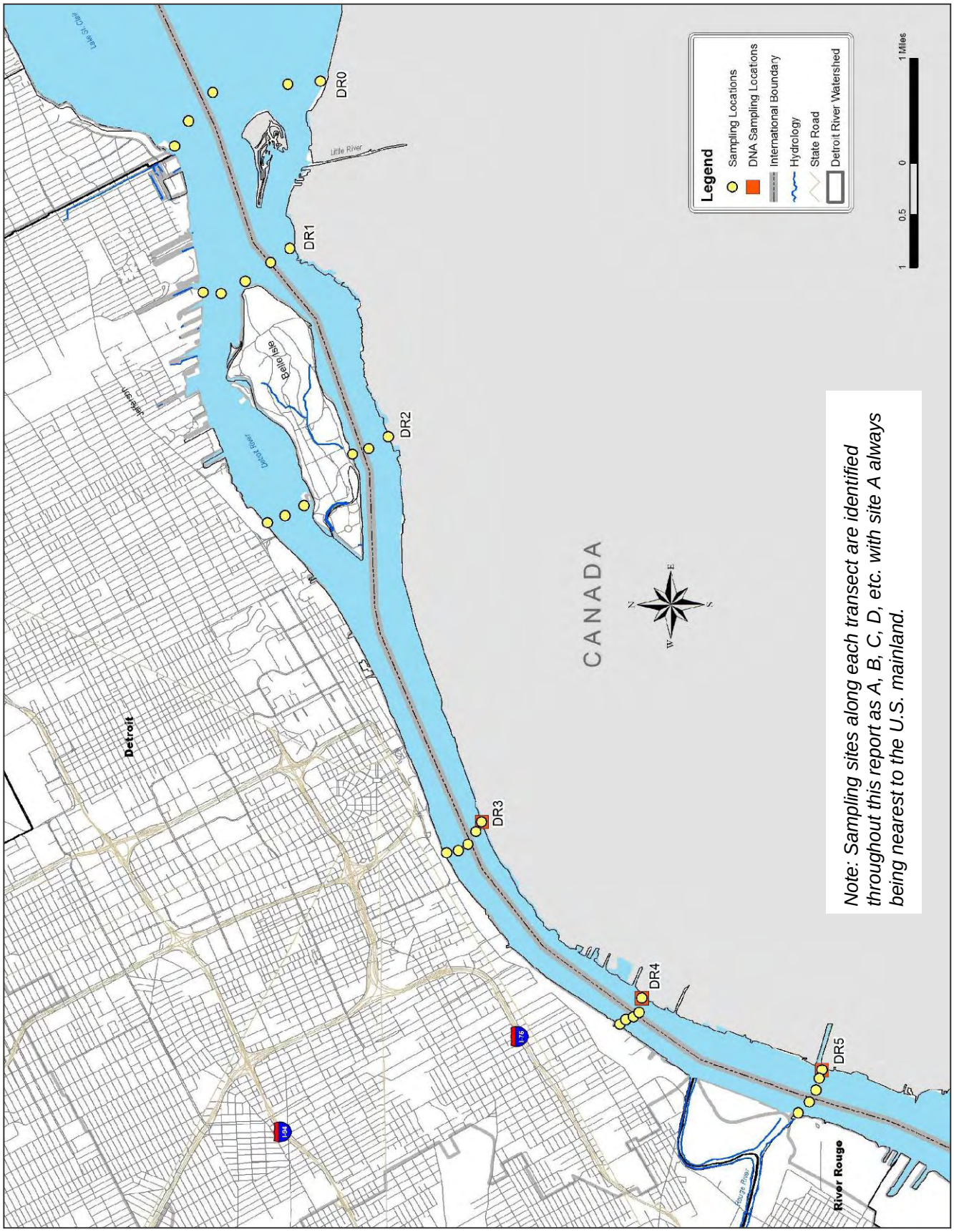


Figure 2b. Lower Detroit River Monitoring Locations

Note: Sampling sites along each transect are identified throughout this report as A, B, C, D, etc. with site A always being nearest to the U.S. mainland.

4.2.2 *E. coli* Results

The *E. coli* analytical data is presented in a variety of manners. Summary statistics are provided in tables and graphs displaying the measure of central tendency (number of samples, median and range) for each monitoring site. The distribution of the data is graphically displayed by weather condition in pie charts that are overlaid on the project maps. Spatial variation from location to location was also considered. The frequency of WQS exceedences is also calculated and displayed. All raw *E. coli* data and the corresponding daily and monthly geometric means are included in Appendix C of Attachment A.

The minimum, maximum, median and average *E. coli* values for each transect are shown in Table 2. Based on the average values, the highest *E. coli* concentrations were found near the outlet of Brownstown Creek (DR8) and in the Trenton Channel (DR7). Elevated maximum values (above 1,000 cfu/100mL) were detected along the Canadian side of Rouge River transect (DR5) downstream to the U.S. side of the Lake Erie transect (DR8).

Table 2. Detroit River *E. coli* Statistics (based on 23 values per transect)

Transect	Sampling Site Groupings†	Country	*E. coli* Results (cfu/100mL)			
			Minimum	Maximum	Median	Average
DR0 – Outlet of Lake St. Clair	A, B, B/C	US	10	30	10	14
	C – E	CA	10	49	10	14
DR1 – u/s of Belle Isle	A – C	US	10	97	10	16
	C/D, D, E	CA	10	56	14	18
DR2 – Belle Isle	A – C	US	10	107	13	23
	D/E, E, F	CA	10	37	13	16
DR3 – Renaissance Center	A – C	US	10	125	10	22
	C/D, D, E	CA	10	119	28	31
DR4 – Fort Wayne	A – C	US	10	130	10	20
	C/D, D, E	CA	10	190	31	46
DR5 – d/s Rouge River	A, B, B/C	US	13	391	23	60
	C – E	CA	16	1,015	37	159
DR6 – d/s Ecorse River DR6 – d/s Turkey Creek	A – E	US	11	2,125	29	151
	F – H	CA	10	1,745	71	231
DR7 – Trenton Channel DR7 – west side of Grosse Ile DR7 – d/s Canard River	A – C	US	10	7,027	19	366
	D – G	US	10	2,584	10	130
	G/H, H, I	CA	10	516	17	75
DR8 – near Brownstown Creek DR8 – u/s of Lake Erie DR8 – u/s of Lake Erie	A*	US	10*	3,000*	80*	403*
	B – F	US	10	6,312	12	264
	G – I	CA	10	87	14	23

*Based on single discrete samples.

†Sampling sites listed as "B/C", "C/D" or "G/H" indicates that *E. coli* values were interpolated between the two sites.

The *E. coli* results were further evaluated based on weather conditions. Wet weather conditions were defined by events with rainfall volumes of 0.2" or more as measured at Detroit Metro Airport over the 30 hour period that preceded the start of a sampling event. Seven wet weather events were sampled during the 23 weeks of sampling.

The distribution of the *E. coli* values for dry and wet weather conditions are depicted in Figures 3a & 3b. From this analysis it is apparent that elevated *E. coli* levels generally occurred during wet conditions.

Next, the daily and 30-day geometric means for each transect were compared to the Michigan WQSs for *E. coli*. This comparison was made for samples taken in U.S. waters only. Of the 230 daily geometric means calculated, the daily target of 300 cfu/100 mL was exceeded seven times (3%). These exceedences occurred at the Rouge River, Ecorse Creek, Trenton Channel, Grosse Ile and Lake Erie transects as shown in Table 3. All seven exceedences were associated with two rain events that measured 2.4" and 0.8" on August 21st and October 2nd, respectively.

The Detroit River exceeded the monthly target of 130 cfu/100 mL in two of 190 30-day geometric means (1%). These exceedences occurred in the Trenton Channel (DR7).

Table 3. Detroit River Water Quality Exceedences (U.S. Waters only)

Transect	Sampling Site Groupings	Daily Geometric Mean			30-Day Geometric Means		
		No. of Values	Exceedences (>300 cfu/100mL)		No. of Values	Exceedences (>130 cfu/100mL)	
			Number	Percent		Number	Percent
DR0 – Outlet of Lake St. Clair	A, B, B/C	23	0	0%	19	0	0%
DR1 – u/s of Belle Isle	A – C	23	0	0%	19	0	0%
DR2 – Belle Isle	A – C	23	0	0%	19	0	0%
DR3 – Renaissance Center	A – C	23	0	0%	19	0	0%
DR4 – Fort Wayne	A – C	23	0	0%	19	0	0%
DR5 – d/s Rouge River	A, B, B/C	23	1	4%	19	0	0%
DR6 – d/s Ecorse Creek	A – E	23	2	9%	19	0	0%
DR7 – Trenton Channel	A – C	23	2	9%	19	2	9%
DR7 – west side of Grosse Ile	D – G	23	1	4%	19	0	0%
DR8 – u/s of Lake Erie	B – F	23	1	4%	19	0	0%
Totals		**230**	**7**	**3%**	**190**	**2**	**1%**

Lastly, a spatial comparison of the Detroit River *E. coli* data was made (See Figures 4a & 4b). For this comparison, the average *E. coli* concentration was calculated at each sampling site for the entire 23 week sampling period. This comparison shows that the highest *E. coli* concentrations were located along the shorelines (both U.S. and Canadian); while the mid-river stations generally had very low *E. coli* concentrations. This trend was most apparent downstream of the Rouge and Ecorse rivers (DR5 & DR6, respectively). With the exception of the shoreline sites, there was little variation of *E. coli* concentrations across the transects. Two exceptions to the higher shoreline *E. coli* concentrations occurred at the outlet of Lake St. Clair (DR0) and across the Trenton Channel (DR7). The river appears to be fairly well mixed horizontally at these transects.

Figure 3a. Detroit River *E. coli* Frequency Distribution – Dry Conditions

Figure 3b. Detroit River Daily *E. coli* Frequency Distribution – Wet Conditions

Figure 4a. Spatial Distribution of Average *E. coli* Concentrations across the Upper Detroit River

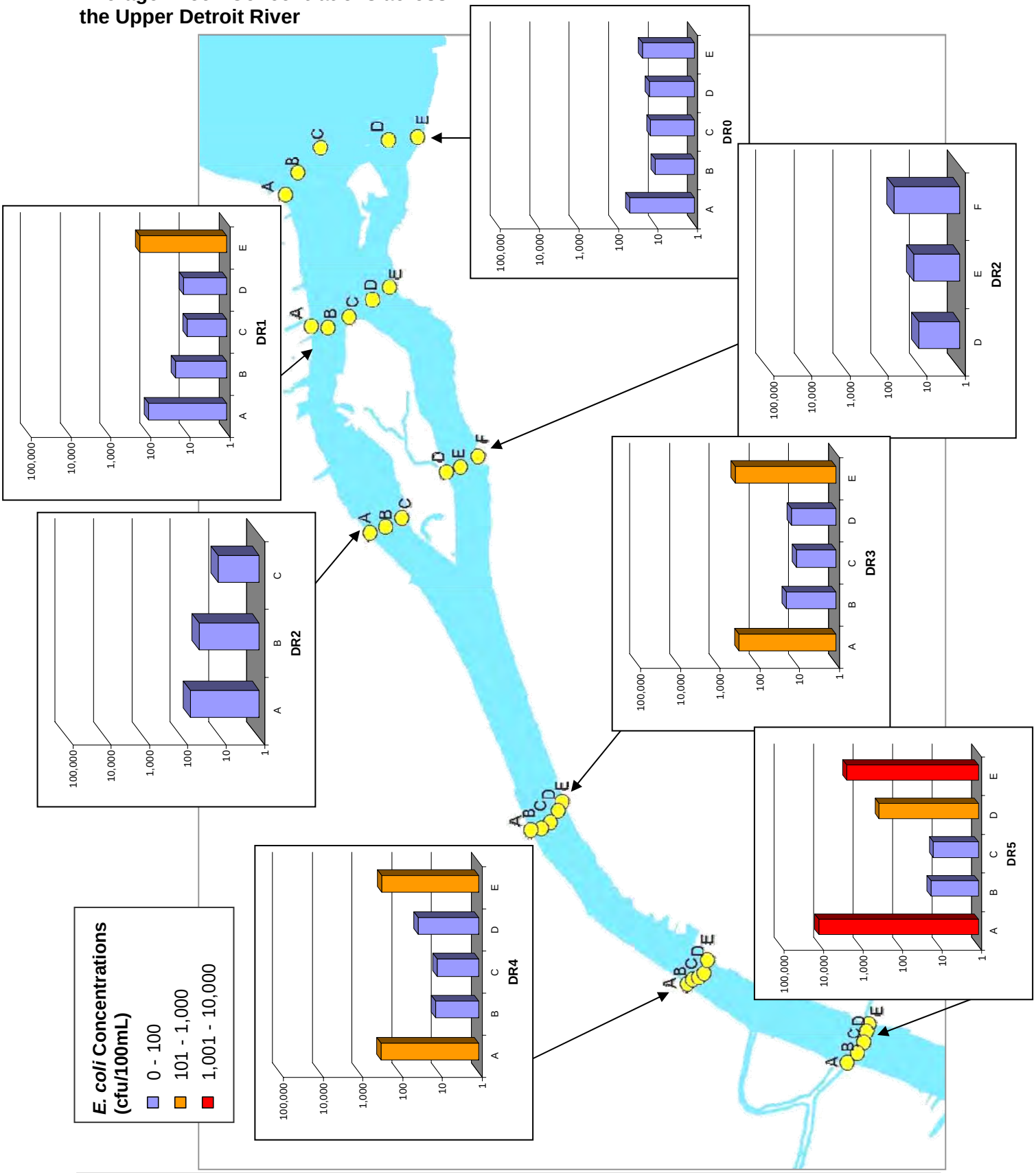
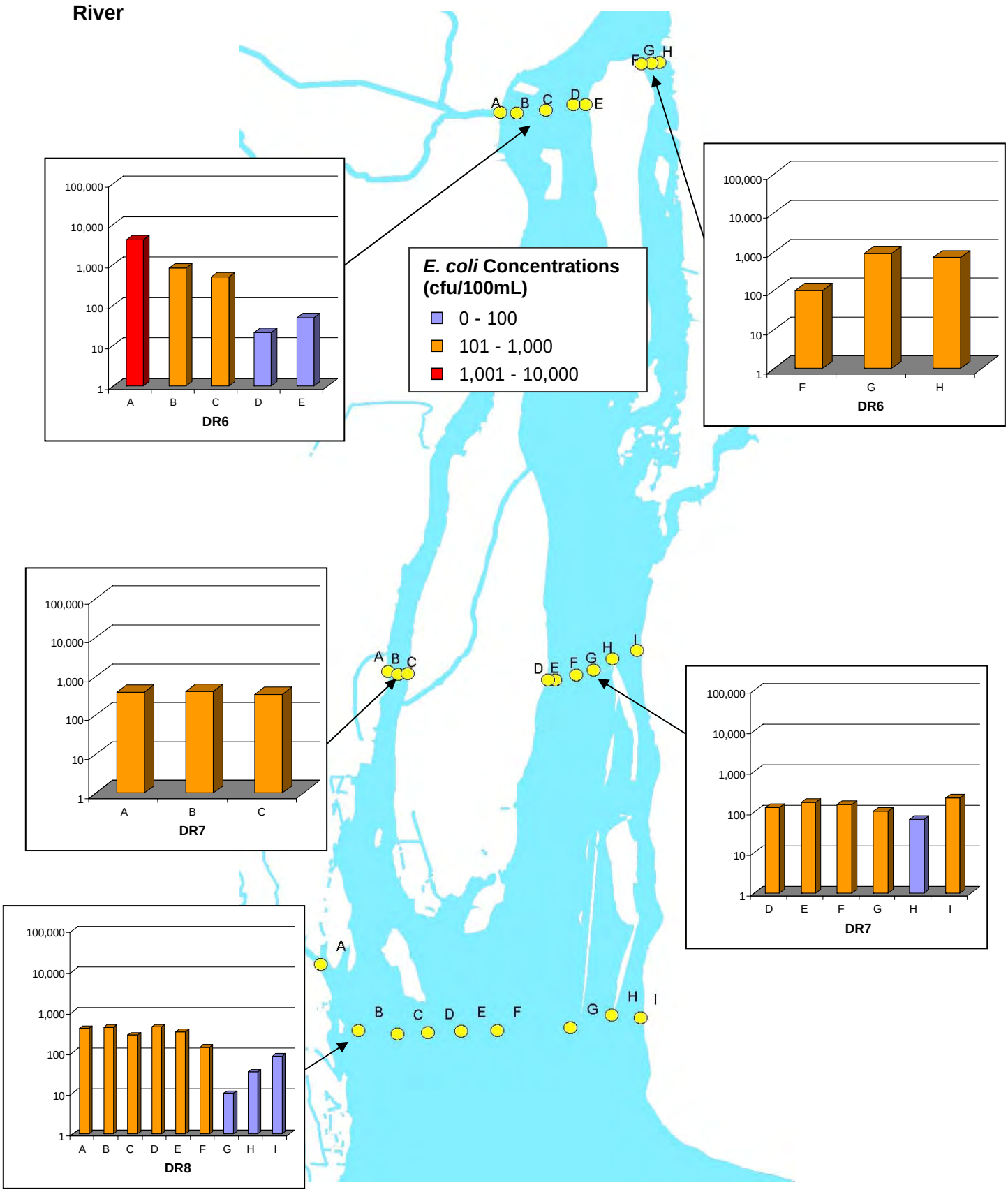


Figure 4b. Spatial Distribution of Average *E. coli* Concentrations across the Lower Detroit River



5.0 SOURCE ASSESSMENT

As mentioned earlier, the Rouge River subwatershed already has an *E. coli* TMDL established, and the Ecorse River subwatershed's *E. coli* TMDL is under development. Therefore, this Section doesn't explicitly address those two subwatersheds. However, it should be noted that each of these Detroit River tributaries contain substantial sources of *E. coli* based on the data that was collected in those subwatersheds in preparation for their TMDLs.

Table 4 shows the land use distribution summarized by subwatershed based on 2000 data obtained from the Southeast Michigan Council of Governments (SEMCOG 2003).

Table 4. Land Use Distribution within the Combined Downriver and North Detroit Subwatersheds

Land Use Category	Combined Downriver		North Detroit	
	Acres	%	Acres	%
Active Agriculture	3,120	5.8	0	0.0
Commercial Office	2,641	4.9	5,253	9.7
Extractive	971	1.8	96	0.2
Grassland	6,213	11.5	360	0.7
Industrial	3,983	7.3	5,818	10.8
Institutional	1,900	3.5	3,686	6.8
Multiple Family	1,273	2.3	3,820	7.1
Outdoor	2,408	4.4	3,540	6.6
Residential With Vacant	0	0.0	13,567	25.1
Single Family	14,519	26.8	12,389	23.0
Transportation	5,972	11.0	3,285	6.1
Under Development	1,027	1.9	0	0.0
Vacant Land	0	0.0	1,834	3.4
Water	395	0.7	35	0.1
Woodland-grassland	9,814	18.1	264	0.5
Total Drainage Area	**54,236**		**53,947**	

The Detroit River watershed is largely urbanized. Potential sources of *E. coli* associated with urban land uses are combined sewer overflows, sanitary sewer overflows, sanitary sewer exfiltration, failing on-site sewage disposal systems, illicit connections and discharges to storm sewers, domestic pets and wildlife.

5.1 NPDES Permits

There are 104 NPDES-permitted discharges in the North Detroit and Combined Downriver watersheds. NPDES permitted discharges to the Ecorse and Rouge River subwatersheds can be found in their respective TMDL reports. The discharge permits include 22 individual permits and 82 certificates of coverage under eight general permits (Attachment C). Many of the NPDES permits authorize the direct discharge of storm water to the Detroit River or tributaries thereof. Storm water collection systems often concentrate numerous sources of *E. coli* from overland runoff and from illegal connections to the storm sewer system. Some permits address

multiple outfalls including sanitary sewer, combined sewer, industrial pretreatment and storm water discharges. A map of the NPDES discharges is also included in Attachment C.

5.2 Wastewater Treatment Plants

Five wastewater treatment plants (WWTP) discharge treated sewage directly to the Detroit River: Detroit WWTP, Wayne County Downriver WWTP, Trenton WWTP, Grosse Ile Township WWTP and the South Huron Valley Utility Authority WWTP. WWTP discharges are considered in compliance with the WQS of 130 *E. coli* per 100 mL if their NPDES permit limit of 200 fecal coliform per 100 mL as a monthly average is met. All five plants are in compliance with the fecal coliform limits in their NPDES permits (note that for the Detroit WWTP a second outfall to the Detroit River is under construction that will allow for disinfection of all excess flows during wet weather once completed).

CSO discharges contain raw, diluted, or partially treated sewage containing *E. coli*, among other pathogens. CSOs are wet weather discharges from combined sewer systems that are designed to carry a mixture of sewage and storm water runoff. During wet weather, the flow in combined sewers may exceed the capacity of pipes or the treatment plant. Once system capacity is reached, raw, diluted, or partially treated sewage bypasses treatment at the WWTP, or is discharged from points in the collection system, and is discharged directly to a receiving water body. CSOs are NPDES permitted discharges for which long-term CSO control programs have been developed and approved by the MDEQ. These plans require the CSO to either be eliminated or provided with treatment to meet water quality standards at times of discharge. Note that CSOs that discharge treated effluent are considered controlled by the MDEQ. Uncontrolled CSOs receive no or insufficient treatment prior to discharge. A map of the CSO outfall locations to the Detroit River is provided in Figure 5.

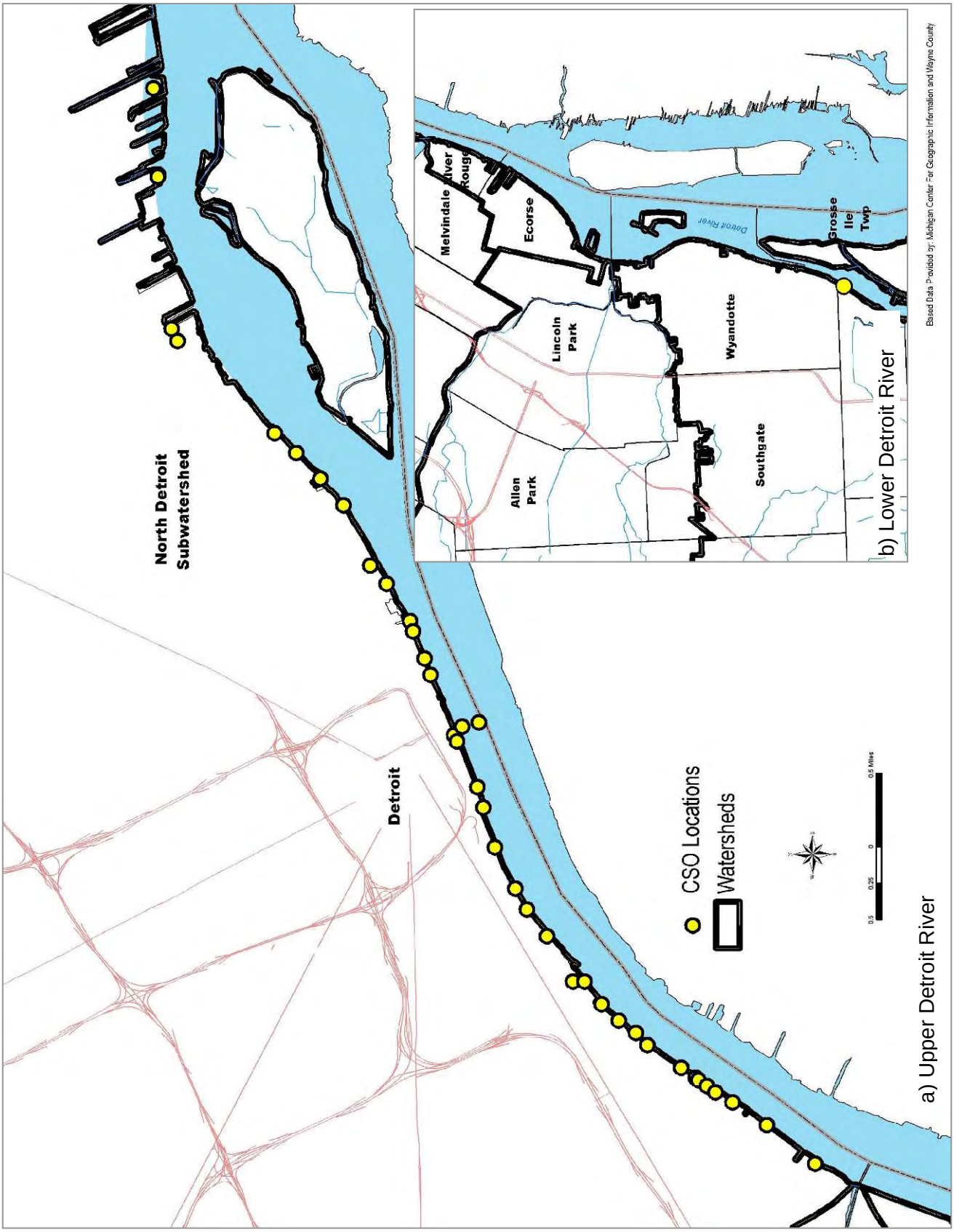
SSOs are wet weather discharges from sanitary sewer systems that contain raw or inadequately treated sewage and some unintended storm water inflow and/or ground water infiltration into the sanitary sewer system. SSOs occur when the capacity of a sanitary sewer system is exceeded. SSOs are illegal events and can occur in any sanitary sewer system served by a wastewater treatment plant.

Both uncontrolled CSO and SSO discharges are considered the primary source of *E. coli* to the Detroit River watershed (please see the Ecorse River TMDL and Rouge River TMDL for sources that are not direct to the Detroit River). The necessary CSO/SSO corrections are organized as follows by the associated wastewater treatment plant.

5.2.1 Detroit Water and Sewerage Department

The Detroit Water and Sewerage Department (DWSD) operates one of the largest WWTPs in the United States with a daily flow of approximately 750 million gallons per day (MGD). It serves approximately three million people in 77 communities that contain both separate sanitary sewer systems and combined sewer systems. The City of Detroit is served almost entirely by a combined sewer system with an estimated service area of 97,240 acres (DWSD 2007). Prior to the implementation of the Long-term CSO Control Plan (LTCP), DWSD had 46 and 32 uncontrolled CSO outfalls along the Detroit and Rouge rivers, respectively, with an estimated annual discharge totaling 20 billion gallons. Seven of the 46 previously untreated CSOs along the Detroit River are now controlled by four treatment facilities (See Attachment D).

Figure 5. Combined Sewer Overflow Outfalls along the U.S. Shoreline of the Detroit River



During the May 1 to October 2, 2007, *E. coli* monitoring period, the Detroit River watershed received 9.9 billion gallons of mostly CSO and some SSO effluent. Approximately 42% (4.4 billion gallons) of that volume was discharged directly to the Detroit River, the rest discharged to the Rouge River. Of the 4.4 billion gallons, approximately 76% of that volume received partial treatment (i.e. skimming, settling and disinfection) prior to discharge to the Detroit River.

The Detroit Water and Sewerage Department conducted shoreline surveys, river transect surveys, and dye studies in 1999 and 2000 to collect data for updating its LTCP (DWSD, 2001). Three formerly untreated CSO discharges to Conner Creek, located in the upstream area of the Detroit River, are an example of the impact untreated CSOs can have on a receiving water. Sampling revealed *E. coli* numbers within Conner Creek of 100,000 cfu/100 mL during one wet weather event as a result of CSO discharges. Dye studies were conducted to evaluate the impact of the Conner Creek CSO discharge on Detroit River water quality. Maximum *E. coli* numbers within the Conner Creek CSO discharge plume in the Detroit River routinely exceeded the partial body contact WQS of 1,000 cfu/100 mL. However, *E. coli* numbers decreased substantially within 100 feet downstream of the Conner Creek confluence due to mixing with the Detroit River (from 18,000 to 950 cfu/100 mL). The Conner Creek CSO discharge plume was generally confined to within 200 to 300 feet of the shoreline near the outfall, but expanded to 500 feet two to three miles downstream of the outfall. Although the discharge from Conner Creek outfall is now controlled, the Connor Creek example demonstrates how uncontrolled CSO discharges can impact the *E. coli* levels in the Detroit River.

5.2.2 Wayne County Downriver WWTP

The Wayne County Downriver Sewage Disposal System serves thirteen communities and consists of major interceptors, retention basins and the Wayne County Downriver WWTP. The Downriver WWTP treats an average flow of 60 MGD, but can treat up to 225 MGD during wet weather. All effluent is disinfected using ultraviolet (UV) light. Major wet weather correction projects were recently completed at the WWTP and in the collection system under a federal consent decree.

There are eight remaining emergency bypass points within the Sewage Disposal System: one at the WWTP discharging to the Detroit River and seven within the collection system discharging to the Ecorse River subwatershed. These outfalls are prohibited to discharge except as defined by the MDEQ in their Consent Order.

The Southgate-Wyandotte Retention Basin (RTB) serves a portion of the combined sewer area within the Wayne County Downriver Sewage Disposal System. The disinfection system for the main outfall (001) at the RTB has recently been upgraded. The other outfall in this system, outfall 002, is a wet weather discharge from the Pine Street Pump Station. This outfall is being evaluated under the current NPDES permit as to its ability to comply with all water quality standards at times of discharge.

During dry weather or low flow events, sewage from the combined portions of Wyandotte and Southgate go to the Wayne County Downriver WWTP. During moderate events, excess flows drain to Pump Station No. 5, receive primary treatment and retention and are discharged back to the WWTP. During heavy events, excess flows from these areas drain to the Pine Street Pump Station where CSOs are potentially discharged to the Detroit River.

5.2.3 Grosse Ile Township WWTP

An ACO was entered between the MDEQ and Grosse Ile in 2001 that called for correction of bypassing at the WWTP to meet state and federal requirements. All corrective actions have been completed except for those on a portion of the redundant interceptor system, which are planned for completion by 2009. Once corrective actions to this interceptor are completed, Grosse Ile will have additional designed capacity in their sanitary sewer system and at the WWTP to meet state and federal SSO requirements (once positively certified by the State).

5.3 Agricultural Practices

There are no Concentrated Animal Feeding Operations (CAFO) in the North Detroit and Combined Downriver subwatersheds. Furthermore, there is no agriculture in the North Detroit subwatershed.

The Combined Downriver subwatershed contains over 3,000 acres of land (5.8%) in agricultural production. Most of the agricultural production is concentrated on the upper Blakely Drain, south of the Wayne County Detroit Metropolitan Airport. Land application of manure, livestock access to streams, and pasture and feed lot runoff can be sources of *E. coli*, but the extent of these agricultural practices is not known for this subwatershed. Regardless, due to the relatively small amount of agricultural land in the Combined Downriver area, agricultural practices are most likely only a minor source of *E. coli* to the Detroit River.

5.4 Illicit Connections and Discharges

The following are key illicit discharge terms and their definitions in the Watershed-Based Storm Water General Permits issued in 1999 and 2002 (MDEQ, 2006):

> *Illicit discharge*: Any discharge (or seepage) to the separate storm water drainage system that is not composed entirely of storm water or uncontaminated groundwater.
>
> *Illicit connection*: A physical connection to the separate storm water drainage system that 1) primarily conveys illicit discharges into the system and/or 2) is not authorized or permitted by the local authority (where a local authority requires such authorization or permit).

Illicit connections to storm sewers can result in the discharge of sanitary wastewater to separated sewer systems and surface waters within the Detroit River watershed. In some cases, an illicit discharge may be a direct discharge of sanitary wastewater from a sanitary sewer improperly discharging to a water body. In other cases wastewater many infiltrate to a storm sewer from a dilapidated sanitary sewer. Illicit discharges of sanitary wastewater are a dry weather source of *E. coli*.

Illicit connections have been documented in the Rouge and Ecorse river subwatersheds, and may be present in the Combined Downriver and North Detroit subwatersheds. Therefore, illicit connections are considered a source of *E. coli* in the subwatershed.

5.4.1 Wayne County Department of Environment (WCDOE)

WCDOE has conducted an extensive illicit connection program under its Illicit Discharge Elimination Plan (IDEP). A total of 1,971 illicit connections and discharges have been identified since 1987. The results of Wayne County's illicit connection detection efforts were evaluated and reported by the Alliance of Rouge Communities in 2007 (ARC 2007). Wayne County identified 44 illicit connections and 222 illicit discharges through investigation of 6,055

complaints between 1999 and 2005. Wayne County identified an additional 1,486 illicit connections and seventeen illicit discharges via dye testing of 3,336 facilities (a 45% failure rate) over a ten year period (1996-2006). Wayne County's IDEP activities have revealed that illicit connections and discharges are a widespread problem in the Wayne County portion of the watershed.

5.4.2 Combined Downriver Subwatershed

The Combined Downriver Subwatershed Inter-Municipality Committee completed a Watershed Management Plan (WMP) in 2005 that included the results of field inventories at 78 locations on 28 different drains in the Combined Downriver subwatershed. The WMP noted possible illicit connections at some of those field inventory locations (CDWIC 2005).

5.4.3 City of Detroit

The City of Detroit operates a small separate sewer system that primarily serves Belle Isle. Since the majority of the island is comprised of parkland and there are very few commercial and no residential buildings on the island, there is a very low probability that illicit connections exist in Detroit's separate sewer system (DWSD 2007).

5.5 On-Site Sewage Disposal Systems (OSDSs)

Failing OSDSs can lead to *E. coli* delivery to the Detroit River when the OSDS is located adjacent to a water body, is seeping into a storm sewer, or has been intentionally or unintentionally connected to a storm sewer. There are no accurate records of the current number of active OSDSs in the Detroit River watershed; however, the Wayne County Health Department estimates that there are approximately 10,000 OSDSs in the County (WCDOE 2007).

The active OSDSs in Wayne County are concentrated in Huron, Sumpter, and Van Buren Townships which are primarily outside of the Detroit River watershed. Active OSDSs are present to a lesser degree in the Rouge River subwatershed in the communities of Canton, Northville, and Plymouth Townships. Some active systems are scattered throughout unsewered areas of Romulus (Downriver, Ecorse and Rouge watersheds), Taylor (Ecorse and Downriver watersheds), Redford and Livonia (Rouge subwatershed), and Brownstown Township (Downriver subwatershed). No active OSDSs are suspected to be located in the North Detroit subwatershed.

Since the number of OSDSs in the Combined Downriver and North Detroit subwatersheds is likely very small, failing OSDSs are probably only a minor source of *E. coli* in the watershed.

6.0 LOADING CAPACITY DEVELOPMENT

The loading capacity (LC) represents the maximum daily loading of a pollutant that can be assimilated by the water body while still achieving WQS. Calculation of the loading capacity and allocations in the Detroit River are complicated by the fact that the international boundary between the United States and Canada runs down the middle of the river, and the two countries use different criteria for their water quality standards. A U.S. TMDL cannot control *E. coli* sources located in Canada; therefore, for the purpose of this TMDL, a loading capacity will only be developed for the U.S. portion of the Detroit River.

The total Detroit River flow was portioned between U.S. and Canadian waters using Acoustic Doppler Current Profiler data obtained from the U.S. Army Corps of Engineers, Detroit District.

Those data provide the cross-sectional area and flow velocity on the U.S. side of the International Boundary at six of the *E. coli* monitoring transects. The analysis of ADCP data resulted in portioning of 52.3% of the total Detroit River flow to U.S. waters, which is the average of the six monitoring transects (see Section 4.0 for further discussion of flow portioning).

Flows along a gradient of 0% to 100% were partitioned into three flow zones: high, moderate and low (see Attachment B, Figure B1) and *E. coli* loads developed for each zone. The three flow zones are represented by their median flow duration interval and value (e.g., 15% is the midpoint of the high flow zone). *E. coli* loads for the three flow zones were developed to be protective throughout the entire range of flows without being overly protective.

As indicated in the Numeric Target section, the targets for this TMDL are the daily maximum of 300 cfu/100 mL and the 30-day geometric mean of 130 cfu/100mL during the total body contact recreation season and 1000 cfu/100 mL, year-round, based on Michigan's WQSs. The daily maximum of 300 cfu/100mL expressed as a daily load will be used in the development of the loading capacity for U.S. portion of the Detroit River.

Concurrent with the selection of numerical limits, development of the LC requires identification of the critical conditions. The critical conditions are the set of environmental conditions (e.g., flow) used in developing the TMDL that have an acceptably low frequency of occurrence. The total daily load allocations must be less than the LC during the occurrence of the critical conditions. The critical conditions for the applicability of the WQSs in Michigan are given in Rule 323.1090 (Applicability of WQS). Rule 323.1090 requires that the WQS apply at all flows equal to or exceeding the water body design flow. In general, the lowest monthly 95% exceedence flow is used as the design condition for developing pollutant loadings.

As described further in Section 7.0, this TMDL provides allowable *E. coli* loadings under three flow conditions: high, moderate and low. The low flow category includes the 95% exceedence flow condition.

For the Detroit River, the loading capacity for the U.S. waters is calculated using the following equation.

The LC is equal to:

Criteria x Flow x Y

Where:

Criteria = WQS ($\frac{300cfu}{100mL}$) for U.S. waters

Flow for U.S. waters = total River discharge as shown in Table 11 ($\frac{ft^3}{\sec}$) x 52.3%

Y = conversion factor ($\frac{28{,}317mL}{ft^3} \times \frac{86{,}400\sec}{day}$)

The resultant LCs for the high, moderate and low flows are presented in Section 7.0 (Table 11).

6.1 Margin of Safety

The margin of safety (MOS) is a required element of the TMDL and accounts for uncertainty in the relation between pollutant loads and the quality of the receiving water body. The approach to assigning WLAs and LAs in this TMDL uses daily flow and the numeric WQS criteria of 300 cfu/100 mL, the TMDL target. Allocations are based on the assumption that ambient *E. coli* concentrations in the Detroit River will be below the TMDL targets and WQS attainment will occur when all regulated NPDES sources of *E. coli* meet the target of 300 cfu/100 mL. This is a conservative approach as explained below. The primary source of uncertainty in this TMDL is estimation of CSO and Detroit River discharge volumes and therefore, the *E. coli* loadings associated with these discharges.

The MOS can be incorporated into the TMDL calculation either implicitly within the WLA or LA, or explicitly. For this TMDL, an explicit MOS was calculated as the difference between the median flow exceedence interval value and minimum flow exceedence interval value for each flow category using the approach developed by Cleland (2006) as shown in Table 5. The MOS was calculated using the U.S. daily target of 300 cfu/100 mL for the U.S. portion of the flow (52.3% of the total flow). The explicit MOS approach ensures that allocations will not exceed the load associated with the minimum flow in each flow category. Therefore, the allocations are protective for the full range of flows in each flow category.

Table 5. Calculation of the Margin of Safety (MOS)

Flow Condition	Median Flow Value (cfs)	Low Flow Value (cfs)	Median Load (cfu/day)	Low Load (cfu/day)	MOS (cfu/day)
High	112,960	108,261	8.29E+14	7.95E+14	3.45E+13
Medium	103,637	96,947	7.61E+14	7.12E+14	4.91E+13
Low	91,923	70,423*	6.75E+14	5.17E+14	1.58E+14

*The 95% exceedence flow value was used for the Low flow condition

The MOS for the high, medium, and low flow categories represents 4.2%, 6.5%, and 23.4% of the median loads, respectively. The most critical flow category is the low flow category when the volume of water in the Detroit River that is available for mixing is lowest. *E. coli* loads occurring during flows within the low flow category have the largest impact on *E. coli* concentrations in the Detroit River.

The TMDL also incorporates a conservative approach that adds an implicit MOS not accounted for in the explicit MOS calculation above. *E. coli* survive for a relatively short time in the environment, depending on environmental conditions. However, the load allocation approach assumes no *E. coli* die-off and that the entire allocated load contributes to Detroit River *E. coli* concentrations.

6.2 Tributary Loads

Tributary loads for the Rouge River and Ecorse River are presented in Section 7.0 (Table 11) as their respective loading capacities. An *E. coli* TMDL has been finalized for the Rouge River and an *E. coli* TMDL is currently under development for the Ecorse River (MDEQ 2008). Loading capacities for the mouth of the two tributaries were calculated for their respective TMDLs using flow duration curves and the numeric criteria (300 cfu/100 mL) for the total body contact recreation WQS. Those flow duration curves were used to calculate loading capacities for the flow categories used in this TMDL.

7.0 ALLOCATIONS

The overall LC was allocated into waste load allocations (WLA) for point sources, load allocations (LA) for non-point sources and the margin of safety (MOS), which was discussed in Section 6.1. The allocations described herein refer only to the Combined Downriver and North Detroit subwatersheds. The allocations for the Rouge and Ecorse River subwatersheds can be found in their respective TMDL reports.

The LC is the sum of individual WLAs, LAs, and the MOS. Conceptually, this definition is denoted by the following equation.

$$LC = \sum WLAs + \sum LAs + MOS$$

Where $\sum$ = sum of

Waste load allocations cover *E. coli* loads from permitted point sources including wastewater treatment plant discharges, runoff from industrial sites and discharges from municipal separate storm sewer systems (MS4s). Load allocations include *E. coli* loads from non-point sources not covered under individual or general discharge permits. Runoff from agricultural land is also included in the load allocation category. A detailed description of each allocation follows, along with the rationale behind each allocation. The resultant numeric loads and a summary of the rationale can be found in Tables 11 and 12.

7.1 Waste Load Allocation

Waste load allocations (WLA) were assigned to sanitary wastewater, industrial storm water, and municipal storm water discharges permitted by an individual NPDES permit or general NPDES permit as described in the sections below. A complete list of NPDES permits addressed in this TMDL is included in Attachment C.

7.1.1 Sanitary Wastewater NPDES Permits

There are six NPDES permits that received a WLA for the discharge of sanitary wastewater as shown in Table 6 (Note that the Detroit WWTP and CSOs are both under one permit). Five of these permits are for WWTPs that discharge directly to the Detroit River. WLAs have been assigned to the individual WWTPs using the NPDES plant design flow rates and the WQS of 300 cfu/100 mL. NPDES design flow rates are noted in Table 11.

Table 6. Facilities with WLA for Sanitary Wastewater

Permit/ Certificate of Coverage Number	Facility Name
Individual Permits (for Discharge of Sanitary Wastewater)	
MI0021156	Wayne Co-Downriver WWTP
MI0021164	Trenton WWTP
MI0022802	Detroit WWTP (including Detroit CSOs)
MI0026191	Grosse Ile Twp WWTP
MI0036072	Southgate/Wyandotte CSO RTF
MI0043800	S Huron Valley UA WWTP

Numerous CSOs also discharge directly to the Detroit River and receive WLAs under two individual permits. The CSOs permitted under the Detroit WWTP individual permit received a

WLA based on output from a hydrodynamic model developed and applied by the City of Detroit as part of its CSO long-term control plan. This was the best method for determining the flow at all 46 Detroit WWTP CSOs in lieu of actual discharge data.

The total flow volume was obtained for the model run for the one year rainfall event (1 inch in 1 hour). The flow produced by this rainfall event is of sufficient magnitude to produce WLAs protective under most conditions. Larger rainfall events may result in a higher *E. coli* load to the Detroit River, but the events are too rare to be protective of the WQS on a daily basis. Smaller rainfall events occur more frequently during the total body contact recreation season, but the total CSO volume and *E. coli* load are fairly low for those smaller events. Consequently, those smaller rainfall events may under-emphasize the impact of CSOs on the total body contact recreation WQS.

The Southgate/Wyandotte CSO retention and treatment facility (RTF) received a WLA based on the maximum recorded event discharge volume recorded between March 1, 2003 and April 1, 2008. The reported precipitation total associated with this CSO event was 1.59 inches. The event occurred between February 5th and 12th of 2008.

7.1.2 Industrial Storm Water NPDES Permits

The WLA for industrial storm water permits was assigned based on the anticipated *E. coli* loading from storm water runoff associated with the industrial areas under various flow conditions. The Long Term Hydrologic Impact Analysis (LTHIA) web application developed by Purdue University, available through the Local Government Environmental Assistance Network, was used to approximate *E. coli* loadings associated with industrial storm water runoff. LTHIA is a curve number-based model that uses land use and hydrologic soil group data to predict long term runoff volumes and non-point source pollution from watersheds. Each land use and hydrologic soil combination is assigned an average curve number. The model uses precipitation data to estimate Antecedent Moisture Conditions (AMC) in the soil and the curve number is adjusted according the AMC conditions. Long-term daily precipitation data is used to predict average annual runoff for each curve number assigned to the various land use/soil group combinations. Dust and dirt accumulation in urban areas and its wash off are used to estimate non-point source pollution, including fecal coliform.

With land use, hydrologic soil information and site location information provided by the user and long-term daily precipitation data queried from an Oracle® database, LTHIA generates runoff volumes and non-point source pollution output in tabular and graphical forms. Non-point source pollution is indicated as an average annual load in tabular form and as a function of percent exceedence of the rainfall in graphical form. The user is able to extract the estimated non-point source pollution loading associated with any given rainfall event based on percent exceedence.

The total industrial land use for the Detroit River watershed (minus the Rouge and Ecorse river watersheds) was determined using the 2000 SEMCOG land use data. The existing available soil information (obtained from the Michigan Center for Geographic Information) was identified for all of the industrial land use area using GIS. LTHIA outputs an annual load in million of fecal coliform (MFC) units per year. A conversion factor as shown in the following formula was applied to calculate daily *E. coli* loads from annual fecal coliform loads predicted by LTHIA.

$$E.coli(\frac{cfu}{day}) = \frac{MFC}{year} \times \frac{1year}{365days} \times \frac{1{,}000{,}000FC}{MFC} \times 0.77\frac{E.coli}{FC}$$

The annual fecal coliform load associated with the 30% rainfall exceedance was obtained from the LTHIA output and converted to a daily *E. coli* load as summarized in Table 7.

Table 7. Modeled *E. coli* Loadings from Industrial Land Uses by Soil Type

Model Input		Model Output	
Soil Type*	Area (ac)**	Annual Fecal Coliform (M FC/year)	Annual *E. coli* (cfu/day)
Soil Group A	171.6	56,053	1.18E+08
Soil Group B	736.5	508,293	1.07E+09
Soil Group C	8,574.4	8,147,354	1.72E+10
Soil Group D	312.8	378,191	7.98E+08
TOTAL	**9,795.3**	**9,089,891**	**1.92E+10**

*As defined by the Natural Resource Conservation Service (NRCS 2008)
** Excluding the Rouge River and Ecorse River subwatersheds

The WLA for industrial storm water includes facilities with a general industrial storm water permit and facilities with an individual permit that authorizes the discharge of industrial storm water to the Detroit River or its tributaries (See Table 8).

Table 8. Facilities with WLA under the Industrial Storm Water Permit Category

Permit/ Certificate of Coverage Number	Facility Name
Individual Permits (with authorized Storm Water Discharges)	
MI0000540	BASF-Wyandotte
MI0001724	DECO-River Rouge Plt
MI0026786	US Steel-GL-Zug Island
MI0001791	DECO-Trenton Plt
MI0036846	Detroit Metro Wayne Co Airport
MI0002313	US Steel-GL-Ecorse
MI0002381	Taminco-Riverview
MI0057359	Ford-Woodhaven Stamping Pt
General Permit MIS210000 - Storm Water from Industrial Activities (2-Yr Cycle Watersheds)	
MIS210782	Levy-Brennan Street Dock
MIS210878	Great Lakes Agg-Highland Park
MIS210886	Dolphin Manufacturing
MIS210888	Superior Materials Plt 2
MIS210900	Alken Ziegler-Taylor
MIS210905	Oakwood Custom Coating
MIS210906	Oakwood Metal Fabricating
MIS210910	Jamison Industries Inc
General Permit MIS310000 - Storm Water from Industrial Activities (3-Yr Cycle Watersheds)	
MIS310018	Group B Industries Inc
MIS310040	Control Manufacturing
MIS310041	Best Concrete & Supply
MIS310042	Solutia Inc-Trenton-Plant 2
MIS310050	Hawkins Steel Cartage
MIS310051	Humbug Marina-Gibraltar
MIS310086	Chrysler LLC-Trenton
MIS310090	Levy-Ecorse-Plt 3

Permit/ Certificate of Coverage Number	Facility Name
MIS310091	Taylor Auto Salvage Inc
MIS310094	Summit Industries-Taylor
MIS310095	Saint-Gobain Abrasives Inc
MIS310143	G & J Cartage Co-Taylor
MIS310146	Fritz Enterprises-Taylor
MIS310147	Fritz Enterprises-Brownstown
MIS310183	FPT Hi-Way
MIS310186	Mich ARNG-Taylor Armory OMS8
MIS310189	Phils Auto Parts-Southgate
MIS310209	Suburban Industries-Gibraltar
MIS310210	Trenton Auto Parts
MIS310211	Levy-Penn Landfill
MIS310212	Ash Stevens-Riverview
MIS310216	Doan Companies-Brownstown Plt
MIS310217	Big 4 Auto Parts
MIS310221	Riverview-Land Preserve
MIS310224	Gregory Boat Company-No 1
MIS310225	Gregory Boat Company-No 2
MIS310238	ET US Holdings LLC
MIS310239	Johnson Controls-Taylor
MIS310273	Kreher Wire Processing-Romulus
MIS310276	Ford-Woodhaven Forging Plt
MIS310284	Elkins Machine & Tool-Romulus
MIS310289	Industrial Fab Inc-Rockwood
MIS310292	Waste Mgt of Mi-Area Disposal
MIS310293	Harbor Hill Marina-Detroit
MIS310294	Trenton Forging Co-Trenton
MIS310315	Millcraft SMS Services-Taylor
MIS310316	Contract Freighters-Taylor
MIS310319	Systrand Manufacturing-Trenton
MIS310387	Aztec Manufacturing-Romulus
MIS310419	Landis Machine Shop-Romulus
MIS310428	Detroit Marine-Detroit River
MIS310470	Federal Screw Works-Romulus
MIS310476	Keans Detroit Yacht Harbor
MIS310508	CTS Engineering-Taylor
MIS310510	UPS-Taylor
MIS310518	Apollo Plating Inc-Taylor
MIS310553	CL Rieckhoff Co Inc
MIS310555	Bucks Oil Co Inc
MIS310569	Huron Valley Steel Corp
MIS310599	Prime Industries
General Permit MIS410000 - Storm Water from Industrial Activities (4-Yr Cycle Watersheds)	
MIS410395	Barrett Paving-Romulus
MIS410413	Oakite Products-Romulus
MIS410554	Johnson Controls Inc-Rockwood
General Permit MIS320000 - Storm Water Discharges with Required Monitoring	
MIS320014	Grosse Ile Municipal Airport
MIS320020	Buckeye Terminals-Woodhaven

7.1.3 Individual or General Permits not authorizing Storm Water

After review of the NPDES permits that do not authorize the discharge of storm water as shown in Table 9, the MDEQ determined that the sites covered under these permits are not likely to contain *E. coli* in their discharges. Therefore, the WLA for these discharges is zero.

Table 9. Facilities not authorized to Discharge Storm Water

Permit/ Certificate of Coverage Number	Facility Name
Individual Permits	
MI0002399	DSC-Trenton Plant
MI0003310	Praxair Inc
MI0001953	DECO-Sibley Quarry
MI0038105	Wyandotte Electric Plant & WFP
MI0001775	DECO-Conners Creek Plt
MI0056243	Steel Rolling Holdings LLC
MI0057709	US Steel-GLW-River Basins
General Permit MIG640000 - Wastewater Discharge from Municipal Potable Water Supply	
MIG640222	DWSD-Water Works Park II WTP
General Permit MIG670000 - Hydrostatic Pressure Test Water	
MIG670085	Buckeye Terminals-Woodhaven

7.1.4 Municipal Storm Water NPDES Permits

All Phase I and Phase II MS4 communities and the Michigan Department of Transportation, listed in Table 10, receive a combined WLA. This combined municipal storm water WLA is equal to the balance of the LC after deducting the MOS and all other WLA (Table 11).

Table 10. Agencies with WLA under the Municipal Storm Water Permit Category

Permit/ Certificate of Coverage Number	Facility Name
Individual Permits	
MI0057364	MDOT State-wide MS4
General Permit MIG619000 - Municipal Separate Storm Sewer System (Watershed-Based)	
MIG610017	Romulus MS4-Wayne
MIG610040	Wayne Co MS4
MIG610344	Grosse Ile Twp MS4-Wayne
MIG610345	Riverview MS4-Wayne
MIG610346	Gibraltar MS4-Wayne
MIG610347	Southgate MS4-Wayne
MIG610348	Taylor MS4-Wayne
MIG610349	Wyandotte MS4-Wayne
MIG610352	Ecorse MS-4 Wayne
MIG610353	Huron Charter Twp MS4-Wayne
MIG610354	Woodhaven MS4-Wayne
MIG610355	Sumpter Twp MS4-Wayne
MIG610356	Brownstown Twp MS4-Wayne
MIG610359	Woodhaven-Brownstown PS MS4-Wayne
General Permit MIS040000 - Municipal Separate Storm Sewer System (Six Min. Measures)	
MIS040066	Detroit MS4-Wayne
MIS040073	River Rouge MS4-Wayne
MIS040038	Trenton MS4-Wayne

Table 11. Loading Capacity and Allocation for the Detroit River (excluding the Ecorse and Rouge River Subwatersheds)

	NPDES Permit (if applicable)	Flow Condition		
		High (0% - 30%)	Moderate (30% - 70%)	Low (70% - 100%)
Median Flow Exceedence Frequency		15%	50%	85%
Total River Flow (cfs)		215,986	198,158	175,761
United States Flow Portion - 52.3% of Total River Flow (cfs)		112,960	103,637	91,923
		Daily Load of *E. coli* (cfu/day)		
Loading Capacity (LC) for United States Waters		8.29E+14	7.61E+14	6.75E+14
Margin of Safety (MOS)		3.45E+13	4.91E+13	1.58E+14
Tributary Loads				
Rouge River		4.25E+12	1.66E+12	8.46E+11
Ecorse River		3.27E+11	4.48E+10	1.17E+10
Available LC		7.89E+14	7.10E+14	5.16E+14
Waste Load Allocations (WLA) –				
WLA – Sanitary Wastewater				
Detroit WWTP (930 MGD; 1,439 cfs)	MI0022802	1.06E+13	1.06E+13	1.06E+13
Detroit CSOs (46 outfalls)	"	6.06E+12	6.06E+12	6.06E+12
Wayne County Downriver WWTP (125 MGD; 193 cfs)	MI0021156	1.42E+12	1.42E+12	1.42E+12
Trenton WWTP (6.5 MGD; 10 cfs)	MI0021164	7.38E+10	7.38E+10	7.38E+10
Grosse Ile Twp WWTP (2.25 MGD; 3 cfs)	MI0026191	2.56E+10	2.56E+10	2.56E+10
Southgate/Wyandotte CSO RTF (1 outfall)	MI0036072	3.07E+11	3.07E+11	3.07E+11
S Huron Valley UA WWTP (24 MGD; 37 cfs)	MI0043800	2.73E+11	2.73E+11	2.73E+11
WLA – Industrial Storm Water	MIS210000 MIS310000 MIS410000 MIS320000	1.92E+10	1.92E+10	1.92E+10
WLA – Municipal Storm Water	MIG619000 MIS040000 MI0057364	7.71E+14	6.91E+14	4.97E+14
WLA – Individual or general permits not authorizing storm water excluding WWTPs	MIG640000 MIG670000	0+00E+00	0+00E+00	0+00E+00
Load Allocation (LA) (non-point sources)	None	0+00E+00	0+00E+00	0+00E+00

7.2 Load Allocation

Non-point sources receive a Load Allocation (LA) in a TMDL. For the Detroit River watershed, all drainage areas are considered to be covered under a NPDES permit, including the MS4 general storm water permits; therefore the load allocation is zero.

Table 12. Allocation Rationale Table for the Detroit River *E. coli* TMDL

Allocation	Rationale
Margin of Safety (MOS)	Explicit: Difference between median flow exceedence interval value and minimum flow exceedence interval value for each flow category; MOS calculated using U.S. daily target of 300 cfu/100 mL for U.S. portion of flow (52.3% of total flow) Implicit: Conservative approach
Rouge River	Discharge flow at mouth of river times daily target
Ecorse River	Discharge flow at mouth of river times daily target
Waste Load Allocations (WLA)	
WLA – Sanitary Wastewater	Detroit CSO WLA is based on the 1-year storm event modeled by the City of Detroit (1-inch in 1-hour). Southgate/Wyandotte CSO WLA is based on maximum recorded discharge volume between March 1, 2003 and April 1, 2008. NPDES design flows used for WWTPs.
WLA – Industrial Storm Water	Long Term Hydrologic Impact Analysis (LTHIA) web application was used to approximate *E. coli* loadings associated with industrial storm water runoff. Web-based application developed by Purdue University: http://cobweb.ecn.purdue.edu/~sprawl/LTHIA7/lthianew/tool.htm
WLA – Municipal Storm Water	Available LC from Table 11 minus allocations for individual permits for sanitary wastewater and CSOs, and general industrial storm water permits and individual permits authorizing storm water.
WLA – Individual or general permits not authorizing storm water excluding WWTPs	No allocation – these permits are not considered a source of *E. coli*
Load Allocation (LA) (non-point sources)	No allocation – although agricultural areas exists, they all discharge to areas covered under municipal or industrial NPDES permits

8.0 SEASONALITY

Seasonality in the TMDL is addressed by expressing the TMDL in terms of a total body contact recreation season that is defined as May 1 through October 31 by R 323.1100 of the WQS. It is expected that there is minimal total body contact during the remainder of the year due to cold weather; however, there is a separate WQS maximum of 1,000 *E. coli* per 100 mL for the partial body contact season, which applies year-round. *E. coli* allocations developed to protect during the total body contact season are also expected to assure attainment of the partial body contact standard.

9.0 MONITORING

E. coli was monitored weekly at nine river transects and 56 stations on the Detroit River from May 1 through October 2, 2007. Future monitoring will take place as part of the 5-year rotating basin monitoring as resources allow, and when corrective actions have occurred to suggest that WQS may be met. When these results indicate that the water body may be meeting WQS,

sampling will be conducted at the appropriate frequency (as defined in the Numeric Target section) to determine if the targets are being met.

10.0 REASONABLE ASSURANCE ACTIVITIES

As mentioned earlier, the Rouge River subwatershed already has an *E. coli* TMDL established, and the Ecorse River subwatershed's *E. coli* TMDL is under development. As such, reasonable assurance activities for meeting the WQS in those two subwatersheds are not explicitly discussed herein, as they are described in their respective TMDLs.

The MDEQ regulates point sources of *E. coli* to the Detroit River and its tributaries through the issuance of NPDES individual permits or certificates-of-coverage (COC) for general permits. The daily maximum and monthly geometric mean WQS concentration levels for *E. coli* presented in the numeric target section (e.g., 300 and 130 cfu/100 mL, respectively), or alternative endpoints that assure attainment of the *E. coli* standards (e.g., best management practices, fecal coliform limitations) will be used to establish any necessary NPDES permit requirements that are consistent with this TMDL.

Point source discharges are the most prevalent source for *E. coli* in the Detroit River watershed, including municipal storm sewers, industrial storm water outfalls, and combined sewers. Therefore, water quality attainment can likely be achieved solely through the regulation of point sources.

The following existing organizational structures and activities demonstrate the ability to eliminate or reduce many of the *E. coli* sources in the Detroit River watershed, particularly wet weather sources, through NPDES permitting and cooperative watershed-based efforts.

10.1 Industrial Storm Water

Federal regulations require certain industries to apply for an NPDES permit if storm water associated with industrial activity at the facility discharges into a separate storm sewer system or directly into surface water. A storm water permit is not required if storm water does not discharge from the facility or is discharged into a sewer system that leads to a WWTP. The general industrial storm water permits identified in Attachment C (MIS210000, MIS310000, MIS320000 and MIS410000) require that if there is a TMDL established by the MDEQ for the receiving water that restricts a material that could impair or degrade water quality, then the required storm water pollution prevention plan shall identify the level of control for those materials necessary to comply with the TMDL and an estimate of the current annual load of those materials via storm water discharges to the receiving stream.

The State of Michigan began issuing industrial storm water permit coverage in 1994. There are three types of permits available in Michigan: a generic baseline general permit, a generic general permit with monitoring requirements, or a site-specific individual permit. There are approximately 4,000 facilities statewide with storm water discharge authorization, with approximately 265 within the U.S. Detroit River watershed. Michigan's storm water permit authorization requires facilities to obtain a certified operator who will have supervision and control over the control structures at the facility, eliminate any unauthorized non-storm water discharges, and develop and implement a storm water pollution prevention plan for their facility that includes structural and nonstructural control measures. Prior to obtaining permit coverage, applicants must certify that they do not have any unauthorized discharges.

MDEQ staff conducts inspections of a percentage of permitted industrial facilities annually. Inspections are utilized to ensure that facilities comply with the regulations, and they result in a further reduction in unauthorized discharges and illicit connections. Also, as additional facilities obtain industrial storm water permits, more illicit discharges will be eliminated.

General permits for wastewater discharges from municipal potable water supplies (MIG640000) and hydrostatic pressure test waters (MIG670000) require that Michigan's WQS are not violated as a result of the discharge. The MDEQ may require the permittee to provide additional sampling or monitoring as deemed necessary to assure adequate operation of the treatment system.

10.2 Municipal Storm Water

The USEPA's Storm Water Phase II Rules require that all municipalities operating MS4s within urbanized areas obtain municipal storm water permits, unless this requirement is waived by the NPDES permitting authority. Since 2003, municipal storm water has been regulated in Michigan under either one of two general permits: watershed based (MIG619000) or six minimum measures (MIS040000). MS4 communities can elect to apply for coverage under either general permit. If a water body has a TMDL established by the MDEQ for a particular pollutant, the MS4 permittee must complete actions to reduce the pollutant in storm water discharges.

10.2.1 Watershed Based Storm Water Permit (MIG619000)

The USEPA and most water resource professionals advocate holistic and adaptive watershed management approaches for the protection and restoration of aquatic ecosystems by encouraging pollution control strategies that are developed through collaborative partnerships within a hydrologic boundary. Michigan was one of the first states to embrace and help develop the concept of watershed-based general storm water permitting.

The Combined Downriver and North Detroit subwatersheds encompass 12 MS4 communities that have obtained coverage under the Phase II Watershed Based Storm Water General Permit (MIG619000, Table 13). Per the requirements of the watershed-based permit, the Combined Downriver Subwatershed Inter-Municipality Committee (CDWIC) prepared a watershed management plan in 2006 that establishes storm water management goals and actions (CDWIC 2006).

Table 13. Combined Downriver and North Detroit Subwatershed Communities covered under the Watershed-based Permit

Brownstown Twp.	Riverview	Taylor
Ecorse	Romulus	Woodhaven
Gibraltar	Southgate	Wyandotte
Grosse Ile Twp.	Sumpter Twp.	Wayne County
Huron Twp.		

There are numerous county drains in the Combined Downriver subwatershed under the jurisdiction of the WCDOE. Wayne County's coverage under the General Permit applies to its operations, facilities, and functions throughout the approximately 626 square mile county (WCDOE 2004). The Water Quality Division coordinates Wayne County General Permit

activities with watershed communities, counties, agencies, subwatershed advisory groups, and other interested parties.

The communities and other stakeholders in the Ecorse River, Combined Downriver, and Lower Huron River subwatersheds have been working together for several years to manage the area's water resources on a watershed basis and to comply with federal regulations regarding the discharge of storm water. The communities have formed a permanent watershed organization established under the Watershed Alliance legislation (Public Act 517 of the Public Laws of 2004). The Alliance of Downriver Watersheds (ADW) provides services to its members and to the existing Advisory Group for each subwatershed in order to facilitate implementation of the Combined Downriver and Ecorse River watershed management plans.

10.2.2 Six Minimum Measures Storm Water Permit (MIS040000)

The Cities of Detroit, River Rouge, and Trenton have obtained Certificates of Coverage under Michigan's Six Minimum Measures general storm water permit (MIS040000) for MS4 communities. The cities of Detroit and River Rouge are located in the North Detroit subwatershed, while Trenton is located in the Combined Downriver subwatershed. The Six minimum measures general MS4 storm water permit requires permittees to develop and implement a Storm Water Management Program Plan (SWMPP) to reduce the discharge of pollutants from the drainage area to the maximum extent practicable to protect the designated uses of the waters of the state and to satisfy appropriate water quality requirements of the federal and state statutes.

The City of Detroit last updated its SWMPP in April of 2007 (DWSD 2007). Detroit's SWMPP provides a framework for implementation of several pollution prevention BMPs covering the areas of illicit discharge elimination, OSDS operation, public education, public involvement and construction site runoff under its on-going Long Term CSO Control Plan. The City identified 50 municipally-owned storm sewers including 22 outfalls to the Detroit River and its tributaries during a comprehensive investigation (DWSD 2007). All but one of those outfalls is located on Belle Isle and the Belle Isle Bridge. The other is located on the Detroit River shoreline just south of the Belle Isle Bridge. The majority of the City's storm sewers drain parklands and/or roads.

The City of Detroit developed its SWMPP through a coordinated multi-agency effort by representatives from several city departments. Storm water management in the City of Detroit involves many entities including the Water and Sewerage Department, the Recreation Department, the Planning and Development Department, the Department of Public Works, the Building and Safety Engineering Department, the Department of Environmental Affairs, the Detroit Economic Growth Corporation, the Greater Detroit Resource Recovery Authority, the General Services Department and Corporation Counsel. Management and control of storm water discharge will be achieved consistent with the City's efforts to control wet weather discharges from its combined sewer system. Completion of the Long Term CSO Control Plan and SWMPP implementation will minimize water quality impacts to the Detroit River to the maximum extent possible (DWSD 2007).

The City of Trenton has had permit coverage since October 31, 2003. The City of River Rouge has had permit coverage since March 22, 2007, and is thus beginning to implement planned programs. Only about two percent of the City of River Rouge is served by separate storm sewers.

10.2.3 Illicit Discharge Elimination

Under municipal storm water permits, permittees are required to develop and implement illicit discharge elimination plans (IDEPs) to prohibit and effectively eliminate illicit discharges (including discharges of sanitary wastewater) to municipal separate storm water drainage systems (MS4s). Communities throughout the watershed have responded to this permit requirement by implementing various activities including:

- Inspection of storm water outfalls and screening storm water discharges for signs of pollution;
- Enacting a report-a-polluter hotline at the county-level to field pollution complaints;
- Training municipal staff at the county and local level on how to detect illicit discharges; and
- Conducting dye testing of various businesses to ensure they are properly connected to the sanitary sewer system.

The Combined Downriver Watershed Management Plan contains a subwatershed action plan section that details planned activities of the communities participating in the Downriver Watershed Alliance, including the Wayne County Airport Authority and Woodhaven-Brownstown School District. Most of those entities are implementing IDEP activities.

The City of Detroit has planned several IDEP BMPs in its SWMPP including staff training, dry weather outfall screening, outfall inventory, dry weather flow sampling, and correction.

The City of Trenton partially fulfills the IDEP requirements of the Six Minimum Measures permit by conducting dry weather screening of their point source discharges. Half of Trenton's outfalls were inspected in 2006, and no illicit discharges were observed. Trenton is in the process of developing a storm sewer database to more readily track illicit discharges.

The City of River Rouge staff will visually inspect and sample the one known storm water discharge point at least once every five years, and eliminate any illicit discharges or connections found.

The City of Trenton has developed and River Rouge is developing geographic information system (GIS) coverage of their respective storm water drainage systems. These GIS coverages include the location of each known stormwater outfall and the respective receiving water or drainage system.

10.2.4 On-Site Sewage Disposal System (OSDS) Management

Under the MS4 permits, municipalities are required to minimize seepage from OSDSs into their storm water drainage systems. OSDS investigations are conducted within Wayne County at the time a home is sold and in response to complaints from the public. Over the past four to five years, Wayne County has identified between 80 and 100 failing on-site septic systems each year through its inspection program of which 90% to 95% are corrected each year (WCDOE 2007). Between 1999 and 2006, Wayne County inspected 1,007 OSDSs county-wide, identifying 239 failed systems and confirming 225 corrections (ARC 2007).

Trenton and River Rouge have sanitary sewer service for every home and business. Within these cities it is a violation of City Building Code and City Ordinance to develop or use property without connecting to City sanitary sewer. Septic systems are not permitted within either City.

10.2.5 Public Education & Involvement

Under the MS4 permits, municipalities are required to develop a public education plan for the purpose of encouraging the public to reduce the discharge of pollutants in storm water to the maximum extent practicable. In response to the MS4 regulations, Wayne County has instituted a 24-hour pollution complaint telephone line to encourage the public to report illegal dumping and water quality problems. Wayne County has also trained many of their field staff on how to identify illicit discharges in storm sewers. Programs like these help reduce the number of water quality problems in the watershed.

10.3 CSO/SSO Control

CSOs can be corrected by either elimination through sewer separation projects or by providing adequate treatment to meet Michigan WQS at times of discharge. Under the long term control plans (LTCP), it is acceptable to treat CSOs to meet federal and state requirements that include, as one significant goal, protecting public health. CSO treatment often involves solids settling, skimming floatable materials and disinfection. CSO discharges are not required to meet federal secondary treatment requirements as they apply to WWTPs, but must still comply with all state water quality standards.

SSOs must be eliminated in accordance with the MDEQ SSO Policy and Clarification Statement.

To date, there has been a great deal of work on CSO control and SSO correction projects that have reduced the volume of untreated wet weather discharge into the Detroit River. Previous and on-going work toward eliminating SSOs and controlling CSOs is summarized below by sewer area.

10.3.1 Detroit Long-term CSO Control Program

The Detroit Water and Sewerage Department (DWSD) continues to make significant progress implementing its LTCP that was submitted in 1996, with approved elements first embodied in its 1997 NPDES permit. Revisions to LTCP projects were made and further included in the 2003 NPDES permit and again in the 2007 NPDES permit. Detroit's LTCP has initially focused on 1) bringing as much wet weather flow to the WWTP as possible during events and treating it to NPDES required primary levels (some treated through full secondary treatment as required by the Environmental Protection Agency CSO policy), 2) using in-system storage in the combined collection system (large flat sewers are available for storage), and 3) by storing/treating priority CSOs on the collection system using retention and treatment basins (RTBs).

The Detroit WWTP (MI0022802) requires that an updated LTCP be submitted by December 1, 2008, that defines the correction projects and associated schedules for the remaining uncontrolled CSOs. The updated LTCP is separated into two parts; one that affects the Detroit River and the other that affects the Rouge River. The specific correction projects and associated schedules will be incorporated into the NPDES permit once approved (either through modification or at the next reissuance). The MDEQ requires that all CSOs be controlled to provide adequate treatment to meet all water quality standards at times of discharge. The State of Michigan does not use less restrictive wet weather water quality standards nor are permittees allowed the option of use-attainability analyses to downgrade designated uses of receiving waters. MDEQ District staff is participating with DWSD staff during its current LTCP update efforts.

Improvements made by the City of Detroit include the following:

- Four CSO Storage/Treatment Facilities along the Detroit River. These include the Conner Creek RTB (30 MG), the Leib Screening & Disinfection Facility (screens and disinfects all CSO flow), the St. Aubin Screening & Disinfection Facility (screens and disinfects) and the 0.3 MG Belle Isle CSO RTB (controls one CSO);
- Eleven storage devices within the collection system. These are inflatable dams in large sewers;
- Additional facilities at the WWTP (i.e. two 180 MGD primary clarifiers, an additional influent pump in PS 2) that have increased its ability to treat combined wet weather flows to primary treatment levels as required by the NPDES permit (capacity now 1.7 billion gallons per day (BGD) as compared to 1.2 BGD prior to submittal of the LTCP);
- Rehabilitation of pump stations and regulators along the Detroit River;
- Completion of the instrumentation/control system that determines CSO flows and discharge times for the remaining untreated CSOs; and
- Completion of, and several revisions to, the collection system hydraulic model used for LTCP planning and specific project design.

The following four CSO retention and treatment facilities along the Detroit River shoreline control seven of Detroit's CSO outfalls:

- 104A Conner Creek Retention Treatment Basin (addresses 3 CSO outfalls);
- 108A Belle Isle RTB (addresses 1 CSO outfall);
- 105A Lieb Screening/Disinfection Facility (addresses 1 CSO outfall); and
- 106A St. Aubin Screening/Disinfection Facility (addresses 2 CSO outfalls).

These facilities have demonstrated the ability to provide adequate disinfection to protect public health and meet Rule 62 across the range of events monitored when properly operated. Attachment D contains a list of uncontrolled CSO outfalls on the Detroit River that are part of the Detroit WWTP combined sewer system.

The MDEQ estimates that the controls implemented to date have resulted in adequate treatment of 85% of the previously discharged untreated CSO volume (annual average of about 20 billion gallons per year – Detroit and Rouge CSOs combined) though only twelve of the 78 outfalls are currently controlled. The City of Detroit, in consultation with the MDEQ, has intentionally addressed the worst CSOs first. Established correction projects will eliminate two untreated CSOs once construction currently underway is completed, seventeen untreated CSOs once the Upper Rouge Tunnel project is completed (plans now in-house for final construction permitting), and an additional five untreated CSOs (for a total of 36 of the 78 CSOs) by the year 2014. It is estimated that the cost of LTCP through 2014 will be approximately 1.1 billion dollars (or more).

10.3.2 Wayne County Downriver WWTP Collection System

As a result of an enforcement action that was brought against Wayne County and the thirteen Downriver Communities, a federal Consent Decree was entered on February 11, 1994. The Consent Decree included a compliance program for sewer rehabilitation and elimination of excess flow, a flow monitoring program, and a schedule for upgrading the WWTP to meet effluent limits. The projects included a regional storage-transport system and relief sewer facilities with sufficient capacity such that the sewage would be transported for treatment in compliance with the requirements of the applicable NPDES permits. The Consent Decree was terminated in 2005 as compliance was achieved.

The following are projects that constitute the various improvements carried out by the communities, the regional system and the wastewater treatment plant.

- Relief Sewers from the Pelham Basin to Jackson Road Pumping Station
- Upper Tunnel & Allen Park Spur
- Eureka Road Relief Sewer
- Lower Tunnel
- Main Tunnel Sewer Allen Road, I-75 to Champaign Road
- Main Tunnel Sewer Pennsylvania Road, Grange Road to Allen Road and Allen Road, Pennsylvania Road to I-75
- Tunnel Pump Station
- Eureka Road Relief Sewer Extension Beech Daly to Middlebelt Road
- Eureka Road Relief Extension Middle Belt to Wahrman Road
- Low Lift Pump Station
- UV Disinfection and Outfall
- Chemically Enhanced Primary Treatment
- Preliminary Treatment: Screening, Grit removal, Disinfection (UV Facility and new outfall)
- Tunnel Dewatering Pump Station
- Southgate-Wyandotte drainage District # 5 Flow Control chamber
- Downriver Storage & Transport Project - System Monitoring Project

There are eight remaining emergency bypass points within the Wayne County Downriver Sewage Disposal System (one at the WWTP to the Detroit River, and seven on the collection system in the Ecorse Creek subwatershed). These are prohibited from discharging except in emergency situations as defined by the MDEQ in their Consent Order.

The Southgate-Wyandotte RTB serves the remaining combined sewer areas within the Downriver system. The existing disinfection system at the RTB Outfall 001 has recently been rehabilitated to provide more effective disinfection. The other outfall in this system is a wet weather discharge from the Pine Street Pump Station (outfall 002). This outfall is being evaluated under the current NPDES permit as to its ability to comply with all water quality standards at times of discharge.

10.3.3 Trenton WWTP Collection System

An ACO was entered between the MDEQ and the City of Trenton in 1996 that called for correction of SSOs to meet state and federal requirements. Fourteen SSO locations were identified in the ACO. The correction program included three new major interceptors, an additional 10.5 MG equalization basin to increase total retention size to 20.0 MG, five new chambers built at the WWTP grounds to control/divert flows, and a WWTP headworks upgrade. Thirteen of the fourteen SSO locations have been eliminated since 1996. The correction project under the ACO has been certified by the MDEQ. As part of this completed project, outfall 002 remains in the system as an emergency discharge to the Detroit River under only the most extreme emergency conditions (designed to the 100 year - 24 hour event for volume, and 25 year - 24 hour event for transport). No further action is required under the ACO for outfall 002.

10.3.4 Grosse Ile Township WWTP Collection System

A 2001 ACO required correction of SSOs (bypassing at the WWTP) to meet state and federal requirements. Downspouts, yard drains, and catch basins connected to the sanitary sewer

system were removed. Sewers and manholes were rehabilitated. Two major interceptors were built to transport peak flow and a 6.5 MG equalization basin was constructed at the WWTP. In addition, a new influent chamber was built at the WWTP grounds to control/divert wet weather flows. All improvements have been completed except for a portion of the redundant interceptor system that is planned to be completed by 2009.

10.3.5 South Huron Valley Utility Authority WWTP Collection System

A 2004 Court Order required the South Huron Valley Utility Authority (SHVUA) to correct SSOs. Prior to this Court Order, a 15-mile long gravity interceptor was constructed in 1993, all seven participating communities removed excess flow sources to stay below their respective contract capacities, and the Odette pump station was built to remove a bottleneck in the collection system. The 2004 Court Order resulted in the construction of a 6 MG equalization basin (designed so that there would be no bypassing at the WWTP at the 25 year - 24 hour event of 3.9 inches of rain in 24 hours). The basin is expandable to 7 MG, if additional capacity is needed.

11.0 PUBLIC PARTICIPATION

The USEPA and MDEQ sponsored two public meetings prior to the release of the draft TMDL. The first meeting was held prior to the onset of the 2007 sampling on March 22, 2007, in conjunction with a regular meeting of the Alliance of Downriver Communities. The second meeting was held on January 30, 2008 on Belle Isle where the 2007 monitoring data and the TMDL development process was discussed. A one-page fact sheet summarizing the monitoring results was prepared and distributed via email prior to this meeting.

A final public meeting was held on July 15, 2008, in the city of Detroit to present the draft TMDL document. Comments were received and addressed.

All meeting dates were advertised on the MDEQ's calendar and meeting announcements were sent via email to numerous stakeholders.

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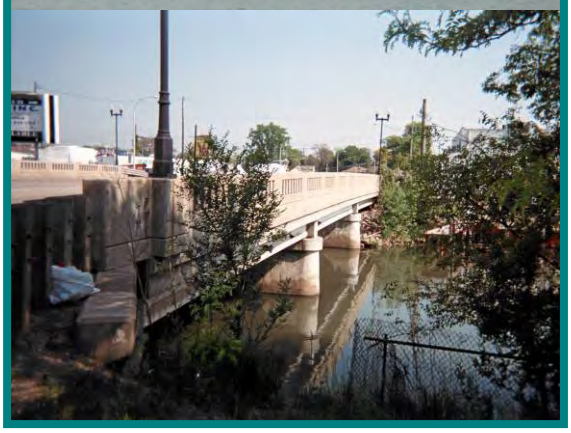
Attachment A
Detroit River & Ecorse River *E. coli* Monitoring to Support TMDL Development
Final Report

Attachment A

DETROIT RIVER & ECORSE RIVER *E. COLI* MONITORING TO SUPPORT TMDL DEVELOPMENT

FINAL REPORT



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Cover Photographs (top to bottom):
Detroit River waterfront in downtown Detroit, Detroit River waterfront in downtown Windsor, Ecorse River outlet in Wyandotte

Acronyms & Acknowlegdements

LIST OF ACRONYMS

CFU	Colony forming units
CSO	Combined sewer overflows
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
ECT	Environmental Consulting & Technology, Inc.
EPA	U.S. Environmental Protection Agency
MDEQ	Michigan Department of Environmental Quality
PCR	Polymerase chain reaction
QAPP	Quality Assurance Project Plan
SMC	Source Molecular Corporation
TMDL	Total maximum daily load
WCDOE	Wayne County Department of Environment
WQS	Water Quality Standard

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Executive Summary

The Detroit and Ecorse rivers were assessed for compliance with Michigan's Water Quality Standards (WQSs) for *Escherichia coli (E. coli)* concentration at various sampling sites. The monitoring for these rivers was prompted by their listings on the State's impaired waters list. Twenty-three consecutive weeks of sampling were performed along both rivers from May to October 2007. Nine transects that ran from Michigan's to Ontario's shoreline were sampled on the Detroit River and nine locations were sampled on the three major tributaries of the Ecorse River. Seven of the twenty-three sampling events occurred during wet weather conditions.

Analysis of the Detroit River daily geometric mean data revealed that the full body contact standard of 300cfu/100mL was exceeded in 3% of the samples. These exceedences occurred during two wet weather events, which had rainfall volumes totaling 2.4 and 0.8 inches. Spatial analysis of the data revealed that flow that passes through the center of the Detroit River (700 feet from the shorelines) almost always had very low *E. coli* concentrations (10 cfu/100mL). Data from the shoreline sites, which were located within 50 feet of the shoreline, was noticeably higher than the rest of the channel at every transect downstream of the Renaissance Center on the US side and at the Belle Isle transect and every transect below downtown Windsor on the Canadian side of the River. The highest shoreline *E. coli* concentrations were found from the Rouge River confluence downstream to Lake Erie.

Analysis of the Ecorse River data revealed that both the North and South Branch of Ecorse Creek rarely met WQSs. On a site by site basis, 76% to 100% of the geometric means exceeded the full body contact standard (300 cfu/100mL) and 52% to 100% of the geometric means exceeded Michigan's partial body contact standard of 1,000 cfu/100mL. The LeBlanc Drain, which is an enclosed storm sewer that drains the middle of the Ecorse River watershed, did not fare any better in terms of *E. coli* concentrations. In fact, 41% of the samples from the LeBlanc Drain exceeded 10,000 cfu/100mL.

The bacterial source tracking analyses indicated that *E. coli* from human source(s) were present in two of the four BST samples analyzed on the Detroit River. These samples were taken during wet weather conditions upstream of the Rouge River confluence near the Canadian shoreline.

The North and South Branches of the Ecorse River and the LeBlanc Drain all tested positive for the presence of *E. coli* from human sources. These results were found throughout each reach mostly during dry conditions. One round of wet weather sampling revealed human sources on the LeBlanc Drain and on the South Branch at Beech Daly Road, as well.

Introduction

PROJECT OBJECTIVES

The primary objective of this monitoring effort was to collect *E. coli* and bacterial source tracking (BST) data to be used in development of a Total Maximum Daily Load (TMDL) for the Detroit and Ecorse rivers. The *E. coli* data was analyzed to determine compliance with the State of Michigan's WQSs, while the BST data was used as an aid in determining the sources of any noted water quality problems.

The project objective was accomplished by collecting routine grab samples from select locations along the Detroit and Ecorse rivers and analyzing them for *E. coli*. Based on the results of the *E. coli* analysis, samples were collected for BST analysis at a subset of locations.

This report is divided into several sections. The remainder of the Introduction section describes the roles of each agency and contractor that worked on the project and provides background information for the two water bodies and their respective drainage areas. The Monitoring Design section discusses the monitoring site locations, analytical methods and sample collection methods. The Discussion & Results section summarizes the results of the *E. coli* and BST tracking analysis by weather conditions and compares the data to WQSs. The Quality Control section describes the degree to which the sampling effort met the data quality objectives described in the Quality Assurance Project Plan. Finally, the Conclusions section summarizes the findings of the sampling effort.

ROLES & RESPONSIBILITIES

The EPA and the MDEQ provided administrative and technical oversight throughout the project. As the prime contractor for the EPA, RTI International provided contractual and administrative support. Environmental Consulting & Technology, Inc. (ECT), a subcontractor to RTI, was responsible for preparation of the Quality Assurance Project Plan (QAPP), sample collection, data analysis and preparation of this report. Paragon Laboratories located in Livonia, Michigan was responsible for the *E. coli* analyses, while Source Molecular Corporation (SMC) located in Miami, Florida performed the bacterial source analyses.

BACKGROUND

The Detroit and Ecorse rivers were placed on the State of Michigan's 303(d) list due to impairment of partial and total body contact designated uses as indicated by exceedances of the *E. coli* WQS in 1998. The Detroit River is also impaired due to PCB and mercury pollution, and the Ecorse River is also listed due to poor macroinvertebrate communities. A biota TMDL for the Ecorse River was approved by the EPA in September 2003 to address the macroinvertebrate impairment.

The designated use rule (R 323.1100 of the Part 4 rules, WQS, promulgated under Part 31, Water Resources Protection, of the Natural Resources and Environmental Protection Act, 1994 PA 451, as amended) states that water bodies are to be protected for total body contact recreation from May 1 to October 31. The target levels for this designated use are the ambient *E. coli* standards established in Rule 62 of the WQS as follows:

> R 323.1062 Microorganisms. Rule 62. (1) All waters of the state protected for total body contact recreation shall not contain more than 130 *E. coli* per 100 milliliters, as a 30-day geometric mean. Compliance shall be based on the geometric mean of all individual samples taken during 5 or more sampling events representatively spread over a 30-day period. Each sampling event shall consist of 3 or more samples taken at representative locations within a defined sampling area. At no time shall the waters of the state protected for total body contact recreation contain more than a maximum of 300 *E. coli* per 100 milliliters. Compliance shall be based on the geometric mean of 3 or more samples taken during the same sampling event at representative locations within a defined sampling area.

Rule 62(2) provides that all waters of the state protected for partial body contact recreation shall not contain more than a maximum of 1,000 *E. coli* per 100 ml with compliance based on the geometric mean of 3 or more samples, taken during the same sampling event, at representative locations within a defined sampling area.

The entire lengths of both rivers are listed as impaired – the Detroit River from Lake Erie upstream to Lake St. Clair, and the Ecorse River from its confluence with the Detroit River upstream to include both the north and south branches of Ecorse Creek and the LeBlanc Drain, an enclosed storm sewer.

WATERSHED DESCRIPTIONS

Detroit River

The Detroit River is located in southeast Michigan and acts as a natural boundary between part of the United States and Canada (See Figure 1). The international boundary between the two counties runs down the River, leaving Belle Isle, U.S. Grassy Island, Zug Island and Grosse Ile on the U.S. side of the River, and Fighting Island, Canadian Grassy Island and Bois Blanc (Boblo) Island on the Canadian side. Approximately 58% of the river's surface area lies within the jurisdiction of the United States, while the remaining 42% lies within Canadian jurisdiction.

Although commonly called a "river", technically the Detroit River is not a river at all, but a connecting channel or strait between Lake St. Clair and Lake Erie. Because of this, the flow rate of the River is virtually the same at its upstream end as its downstream end, unlike a typical river where flows increase markedly from upstream to downstream. The importance of this distinction will be further discussed within the TMDL document, where flow data is incorporated.

The river length is approximately 32 miles from Lake St. Clair to Lake Erie (See Figure 1). The water- surface elevation falls approximately 3 ft within the River, which has an average discharge of about 186,000 ft^3/s (Holtschlag p. 10). The time of travel for water in the River is on average between 19 and 21 hours from the outlet of Lake St. Clair to the river's confluence with Lake Erie (Hamdy p. 449).

The Detroit River receives discharges from numerous stormwater outfalls, industrial point sources, wastewater treatment plants and several combined sewers (during heavy rains). The Rouge River, Ecorse River, Frank & Poet Drain, and Brownstown, Conner, Fox and Marsh creeks all discharge to the River from the U.S. side. The Canard and Little rivers and Turkey Creek discharge to the River from Ontario.

The Detroit River serves as the source of drinking water to 6.2 million people in southeast Michigan and Canada (Holtschlag p. 9). The River also serves as a source of cooling water for numerous industries located in both countries.

The Detroit River watershed covers 434,300 acres (679 square miles) of mostly urbanized land in southeast Michigan, as well as 70,900 acres (111 square miles) in Ontario (Hamdy p. 450). Sixty-two Michigan communities lie partially or completely in the watershed (See Figure 1). The largest land cover category is single family residential at 40% of the U.S. portion of the watershed (See Table 1).

Ecorse River

The Ecorse River drains 27,672 acres (43 square miles) of Wayne County, Michigan and is home to about 160,000 people. There are two primary watercourses within the Ecorse River watershed: the North Branch of Ecorse Creek extends 17 miles in the northern portion of the watershed and the South Branch Ecorse Creek (also known as the Sexton-Kilfoil Drain) extends 13 miles draining the southern portion of the watershed (See Figure 1). In addition to the open watercourses, the LeBlanc Drain, an enclosed storm sewer owned and operated by Wayne County, runs 9.6 miles and drains the central portion of the watershed. As of September 2004, the River was receiving discharges

from 48 permitted point source discharges and numerous storm sewers (ECIC p. 2-1).

There are several cities and a major airport that lie completely or partially within the Ecorse River watershed. These entities are listed below.

- Allen Park
- Dearborn Heights
- Ecorse
- Inkster (small portion)
- Lincoln Park
- Melvindale (small portion)
- Romulus
- Southgate
- Taylor
- Westland
- Wyandotte
- Detroit Metropolitan Wayne County Airport

Land use for the watershed shows that the most prevalent land use type is single-family residential at 46% of the watershed. Another 43% of the watershed is divided between the following land use types: commercial, industrial, grasslands, institutional, transportation and woodlands and wetlands at less than 10% each (See Table 2).

Figure 2 depicts the distribution of land use throughout the Detroit and Ecorse river watersheds.

Table 1. Detroit River Watershed Land Use Data for Michigan (SEMCOG 2000)

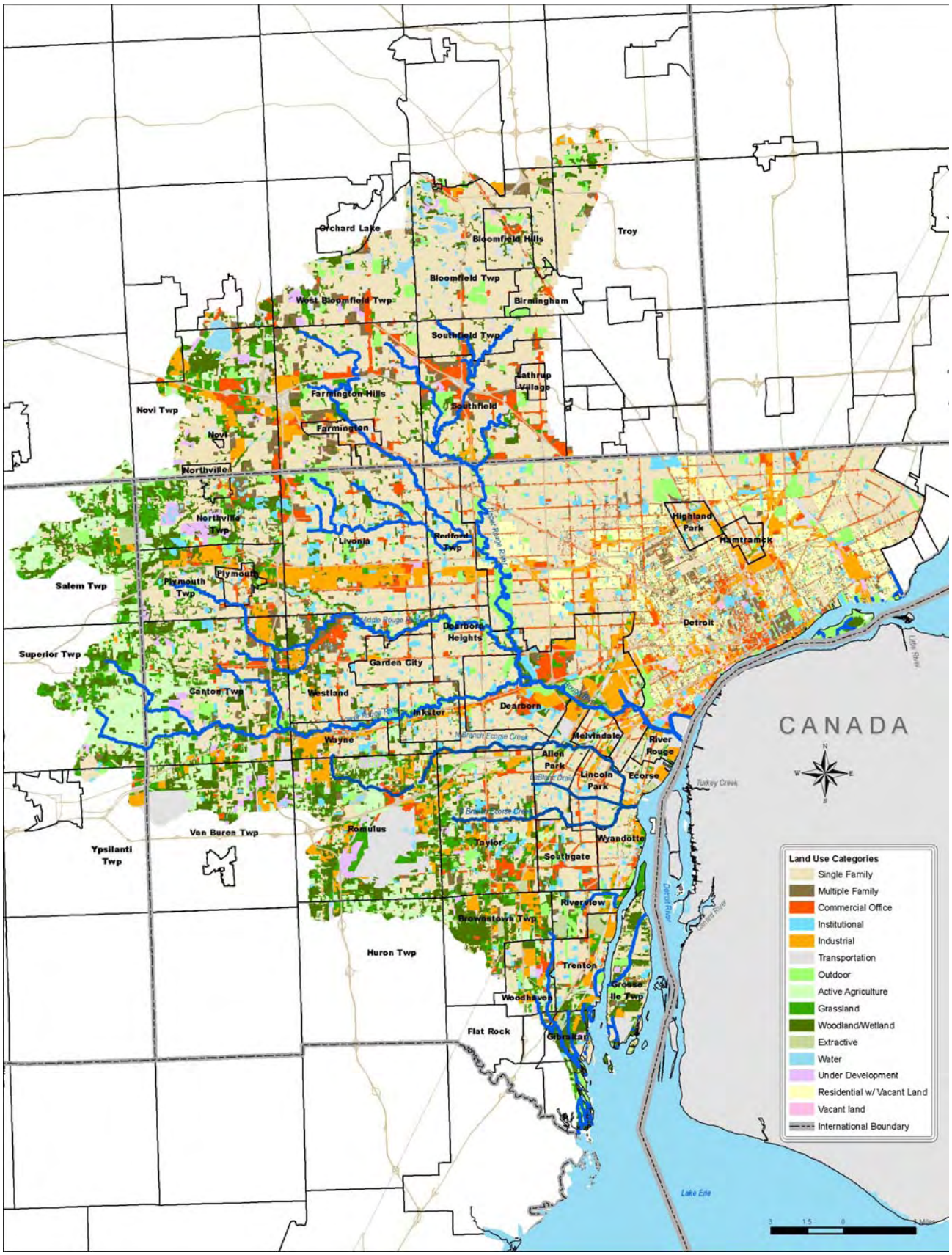
Land Cover	Acres	%
Active Agriculture	19,515	4.5%
Commercial Office	28,886	6.7%
Extractive	1,618	0.4%
Grassland	26,698	6.1%
Industrial	29,169	6.7%
Institutional	15,737	3.6%
Multiple Family	17,977	4.1%
Outdoor	21,266	4.9%
Residential With Vacant	21,049	4.8%
Single Family	172,969	39.8%
Transportation	28,766	6.6%
Under Development	5,227	1.2%
Vacant Land	2,584	0.6%
Water	3,989	0.9%
Woodland - Grassland	38,850	8.9%
Total	434,300	

Table 2. Ecorse River Watershed Land Use Data (SEMCOG 2000)

Land Cover	Acres	%
Active Agriculture	1,265	5%
Commercial Office	1,980	7%
Extractive	139	1%
Grassland	2,012	7%
Industrial	2,017	7%
Institutional	1,341	5%
Multiple Family	543	2%
Outdoor	727	3%
Residential With Vacant	0	0%
Single Family	12,773	46%
Transportation	2,315	8%
Under Development	263	1%
Vacant	0	0%
Water	152	1%
Woodland - Grassland	2,144	8%
Total	27,672	

Figure 1. Project Area Map

Figure 2. Land Use Distribution (SEMCOG 2000)



MONITORING SITE LOCATIONS

Detroit River

The primary factors used to determine the monitoring locations on the Detroit River were the location of known combined sewer outfalls, the location of the international boundary and available budget. Even though the MDEQ and EPA do not have jurisdiction in Canadian waters, an assessment of the Detroit River would have been incomplete if Canadian waters were not considered. Therefore sampling occurred shore to shore across the international boundary. Nine monitoring transects were placed roughly evenly spaced over the course of the River, with one placed at the most upstream and downstream ends of the River.

There were multiple sampling sites associated with each transect. The number of sampling sites was based on the width of the river. Three to nine sampling sites were roughly evenly spaced across the river. The left and right most sites were generally within 50 feet of the shorelines. Sampling site "A" always corresponds to the left most sample nearest to the US mainland. The sampling transects and their corresponding sampling sites are described in Table 3 and Figures 3a and 3b.

Table 3. Detroit River Transect Descriptions (listed upstream to downstream)

Transect	Sampling Sites	Transect Description
DR0	A – E	Outlet of Lake St. Clair and upstream end of Windmill Pointe Park and upstream of Peche Island
DR1	A – E	Upstream of Belle Isle and downstream of Little River
DR2	A – C	Scott Middle Ground near the Detroit Boat Club, north side of Belle Isle
DR2	D – F	Fleming Channel, south side of Belle Isle
DR3	A – E	Downstream of the GM Renaissance Center
DR4	A – E	At Fort Wayne
DR5	A – E	Downstream of the Rouge River cut-off channel and Zug Island
DR6	A – E	Downstream of the Ecorse River
DR6	F – H	North end of Fighting Island to the downstream of Turkey Creek
DR7	A – C	Trenton Channel near Elizabeth Park
DR7	D – I	Grosse Ile to downstream of the Canard River
DR8	A	Near Brownstown Creek outlet
DR8	B – I	Upstream of Lake Erie and downstream of Celeron Island to the Canadian mainland

Ecorse River

Several factors were considered during selection of the monitoring sites for the Ecorse River including: flow conditions (the Detroit River keeps the Ecorse River and the LeBlanc Drain surcharged with water for several miles inland), drainage characteristics, field crew safety during sampling and available budget. Ten sites were selected: four sites on the North Branch, three sites on the South Branch, two on the LeBlanc Drain and one on the main stem of the River near its outlet to the Detroit River. The sampling sites are depicted in Figure 4 and in Table 4.

At monitoring locations EC8 and EC10, the LeBlanc Drain runs within twin, side by side concrete pipes. Since it did not appear that these twin pipes were hydraulically connected, both pipes were sampled at each of these locations. An illegal sanitary sewer connection from a mobile home park to the LeBlanc Drain was discovered by the Wayne County Department of Environment in mid June. This connection was located just upstream of monitoring station

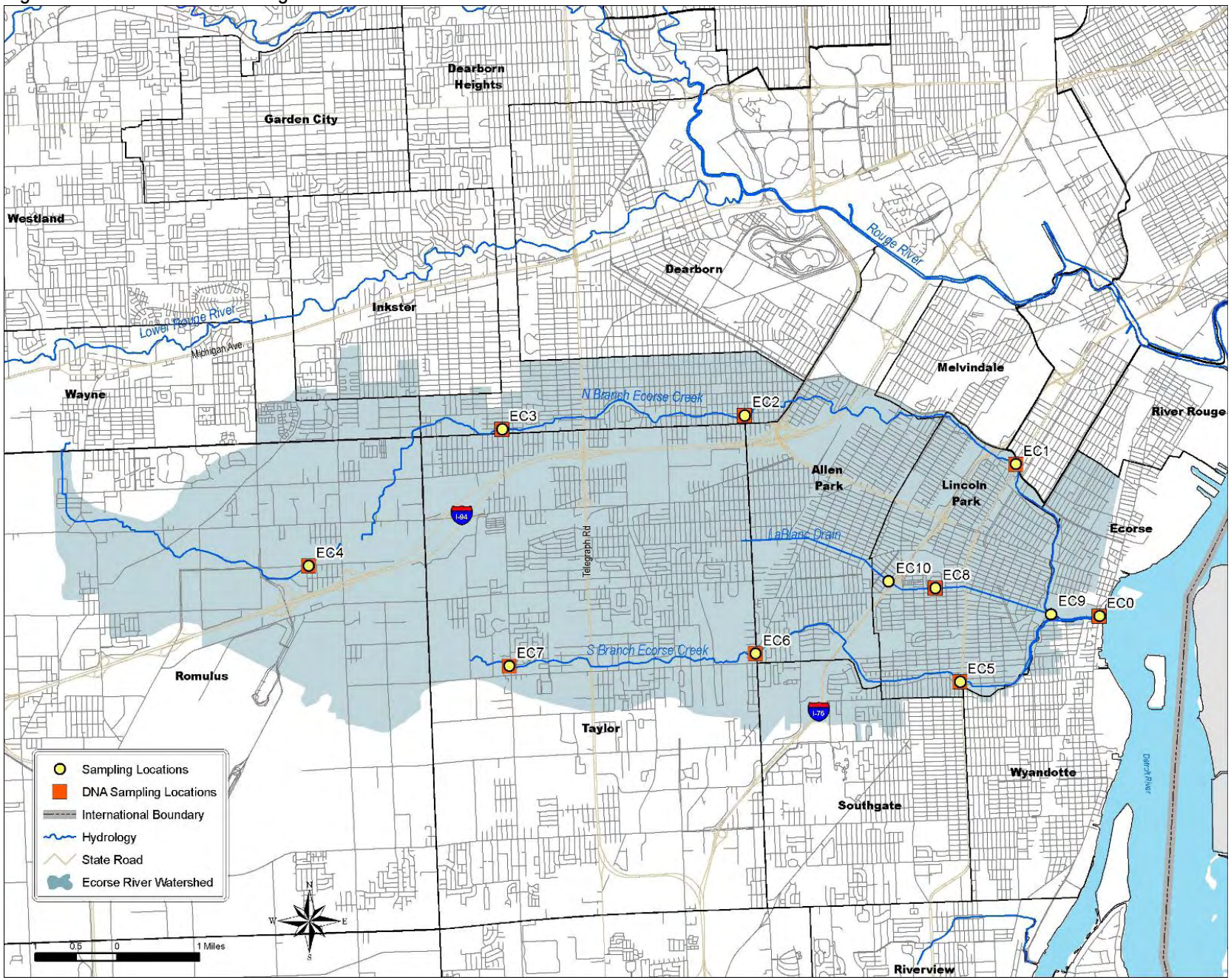
Figure 3a. Upper Detroit River Monitoring Locations

Note: Sampling sites along each transect are identified throughout this report as A, B, C, D, etc. with site A always being nearest to the US Mainland.

Figure 3b. Lower Detroit River Monitoring Locations

Note: Sampling sites along each transect are identified throughout this report as A, B, C, D, etc. with site A always being nearest to the US Mainland.

Figure 4. Ecorse River Monitoring Locations



EC8B. Upon this discovery, sampling was halted at EC8B, and station EC10 was added about a half of a mile upstream of EC8.

A more detailed description of both the Ecorse and Detroit river monitoring locations, which includes latitude and longitude information, aerial maps and site photographs, is contained in Appendix A.

Table 4. Ecorse River Monitoring Site Descriptions (listed by branch upstream to downstream)

Site ID	Site Description
EC0	Main stem at W. Jefferson Ave.
North Branch of Ecorse Creek	
EC4	at Smith Street
EC3	at Beech Daly Road
EC2	at Pelham Road
EC1	at Fort Street
South Branch of Ecorse Creek	
EC7	at Beech Daly Road
EC6	at Pelham Road
EC5	at Fort Street
LeBlanc Drain	
EC10A EC10B	South pipe at Hartwick Road and N. Wayne Street North pipe at Hartwick Road and N. Wayne Street
EC8A EC8B	South pipe at Capitol Road and Howard Street North pipe at Capitol Road and Howard Street
EC9	Drain outlet at Council Pointe Park

SAMPLING FREQUENCY & METHODS

All monitoring sites were sampled on a weekly basis from May 1, 2007 through October 2, 2007. Two exceptions to the weekly sampling occurred at sites EC0 and EC9. These sites were only sampled when flow was present. Each site was visually inspected during wet conditions and if positive flow was observed, then a single sample was collected. In addition, there were a few occasions during the sampling period that flow was not observed at sites EC2, EC4, EC5 and EC10. These sites were not sampled when flow was absent.

For the North and South Branches of Ecorse Creek, three grab samples were taken at every monitoring station. Samples were collected across the stream width at the 25th, 50th and 75th quartile locations. For the LeBlanc Drain a single grab sample was collected at each site. For the Detroit River, a single grab sample was collected at each sampling site across the transect.

After several weeks of sampling, the analytical data was reviewed by the project team for the purpose of determining where to collect the samples for BST analysis. Factors considered were previous *E. coli* concentrations, weather conditions and budget. Locations that frequently had elevated *E. coli* concentrations, regardless of location, were targeted for BST testing based on weather conditions. Initially only ten BST analyses were budgeted in the project scope, but in September 2007 additional funding was released by the EPA. This allowed for approximately 20 additional BST analyses for the project area. The resulting BST locations are depicted in Figures 3a, 3b and 4.

A complete description of the sampling methodology can be found in the Quality Assurance Project Plan, which is included as Appendix B.

ANALYTICAL METHODS

Three analytical methods were utilized during this project – one traditional microbiological method was used to quantify the samples for *E. coli* density and two genetic microbial source tracking methods were used to identify the sources of the *E. coli* bacteria. *E. coli* enumeration was conducted using EPA method 1103.1. Multiple dilutions of each sample were analyzed yielding results between 10 and 360,000 colony forming units/100mL (cfu/100mL).

The genetic methods, the Human *Enterococcus* ID™ and the Human *Bacteroidetes* ID™, used the same process (polymerase chain reaction) to detect biomarkers for human contamination. Results for both tests were reported as either "positive" or "negative" for a biomarker that is found only in bacteria from the human gastrointestinal system. *Bacteroidetes* organisms will not survive long outside of the host organism, but are found in higher numbers in fecal samples. Therefore, a "positive" for the Human *Bacteroidetes* ID™ test indicates the presence of recent and likely a geographically close source of fecal pollution from human source(s). *Enterococcus* organisms survive longer than *Bacteroidetes*, but are found in fewer numbers in fecal samples. Therefore, presence of the human *Enterococcus* biomarker indicates *E. coli* contamination from a human source that may have occurred days prior and/or a longer distance upstream of the sampling point. A “positive” result for either test indicates the presence of *E. coli* from human source(s).

It should be noted that false negatives can occur with either test when low numbers of the target organisms are present.

Results & Discussion

The *E. coli* analytical data is presented below in a variety of different manners. Summary statistics are provided in tables and graphs displaying the measure of central tendency (number of samples, geometric mean and range) for each monitoring site. The distribution of the data is graphically displayed by weather condition in pie charts that are overlaid on the project maps. Spatial variation from location to location was also considered. The frequency of WQS exceedances is also calculated and displayed. On the Detroit River, only data from U.S. waters was used in the comparison to the WQSs.

Approximately 500 samples were collected and analyzed for *E. coli* from the Ecorse River, while 1,300 samples were collected and analyzed from the Detroit River. A total of thirty-one BST samples were collected and analyzed: twenty-seven samples from the Ecorse River and four from the Detroit River. The sampling dates and all analytical results are included in Appendix C.

DETROIT RIVER *E. COLI* CONCENTRATIONS

For the purpose of calculating geometric mean values, results for the Detroit River transects were divided into two or three groups based on the location of islands in the River and by the location of the international boundary. This resulted in separate geometric means for samples collected in US and Canadian waters. A minimum of three *E. coli* values were used for the geometric mean calculation per the Michigan WQSs. For transects that only had two samples, a third value was obtained by interpolating between the nearest two samples. For instance, at transect DR0 only two samples, A and B, were collected in US waters (See Figure 3a). A third value was estimated by interpolating between sampling site B and C. These three values were used to calculate the geometric mean for the US portion of the transect.

At the transect along Grosse Ile, three daily geometric means were calculated: one for the Trenton Channel, one for the west side of Grosse Ile in US waters and one for west side of Grosse Ile for samples in Canadian waters. Upstream of Lake Erie along transect DR8, sample A was handled separately because it was located away from the rest of the samples, near the outlet of Brownstown Creek.

The minimum, maximum, median and average geometric mean values for each transect are shown in Table 5. Based on the average values, the highest *E. coli* concentrations are found in the near Brownstown Creek (DR8) and the Trenton Channel (DR7). Very high maximum geometric values (above 1,000 cfu/100mL) were detected from the Canadian side, directly across from the Rouge River (transect DR5) downstream to the US side of the Lake Erie (DR8) transect.

The daily geometric mean values were further evaluated based on weather conditions. Wet weather conditions were defined by events with rainfall volumes totaled 0.2" or more over the 30 hour period that preceded the start of a sampling event. Seven wet weather events were sampled during the twenty-three weeks of sampling.

The distribution of the daily geometric mean values for dry and wet weather conditions are depicted in Figures 5a & 5b. From this analysis it is apparent that elevated geometric means generally occurred during wet conditions. Wet weather sources typically include contaminated stormwater runoff, untreated combined sewer overflows and sanitary sewer overflows. A detailed analysis of the possible sources of *E. coli* in the Detroit River watershed will be assessed in the upcoming TMDL document.

Table 5. Detroit River Geometric Mean Statistics (based on 23 values per transect)

Transect	Sampling Site Groupings	Country	Daily Geometric Means (cfu/100mL)			
			Minimum	Maximum	Median	Average
DR0 – Outlet of Lake St. Clair	A, B, B/C	US	10	30	10	14
	C – E	CA	10	49	10	14
DR1 – u/s of Belle Isle	A – C	US	10	97	10	16
	C/D, D, E	CA	10	56	14	18
DR2 – Belle Isle	A – C	US	10	107	13	23
	D/E, E, F	CA	10	37	13	16
DR3 – Renaissance Center	A – C	US	10	125	10	22
	C/D, D, E	CA	10	119	28	31
DR4 – Fort Wayne	A – C	US	10	130	10	20
	C/D, D, E	CA	10	190	31	46
DR5 – d/s Rouge River	A, B, B/C	US	13	391	23	60
	C – E	CA	16	1,015	37	159
DR6 – d/s Ecorse River DR6 – d/s Turkey Creek	A – E	US	11	2,125	29	151
	F – H	CA	10	1,745	71	231
DR7 – Trenton Channel DR7 – west side of Grosse Ile DR7 – d/s Canard River	A – C	US	10	7,027	19	366
	D – G	US	10	2,584	10	130
	G/H, H, I	CA	10	516	17	75
DR8 – near Brownstown Creek DR8 – u/s of Lake Erie DR8 – u/s of Lake Erie	A*	US	10*	3,000*	80*	403*
	B – F	US	10	6,312	12	264
	G – I	CA	10	87	14	23

*Based on single analytical values, not daily geometric means
Sampling sites listed as "B/C" indicates that *E. coli* values were interpolated between sites B and C.

Next, the daily and 30-day geometric means for each transect were compared to the Michigan WQSs for *E. coli*. This comparison was made for samples taken in US waters only. Of the 230 daily geometric means calculated, the WQS was exceeded seven times. These exceedences occurred at the Rouge River, Ecorse Creek, Trenton Channel, Grosse Ile and Lake Erie transects as shown in Table 6. All seven exceedences were associated with two rain events that measured 2.4" and 0.8" on August 21st and October 2nd, respectively[1].

The Detroit River 30-day geometric means exceeded Michigan's monthly WQS two of 190 times. These exceedences occurred in the Trenton Channel.

Lastly, a spatial comparison of the Detroit River *E. coli* data was made (See Figure 6a & 6b). For this comparison, the average *E. coli* concentration was calculated at each sampling site for the entire twenty-three week sampling period. This comparison shows that the highest *E. coli* concentrations were located along the shorelines (both US and Canadian); while the mid-river flows generally had very low *E. coli* concentrations. This is most apparent downstream of the Rouge and Ecorse rivers (DR5 & DR6, respectively). Absent of the direct influence of the inland rivers, there is less variation of *E. coli* concentrations across the transects, although shoreline samples still tend to be higher. Two exceptions to this occur at the outlet of Lake St. Clair (DR0) and across transect DR7. The River appears to be fairly well mixed horizontally at these transects.

[1] Rainfall amounts were summed over the 30 hour period prior to the start of sampling, as measured at Detroit Metro Airport.

Table 6. Detroit River Water Quality Exceedences (US Waters only)

Transect	Sampling Site Groupings	Daily Geometric Mean			30-Day Geometric Means		
		No. of Values	Exceedences (>300 cfu/100mL)		No. of Values	Exceedences (>130 cfu/100mL)	
			Number	Percent		Number	Percent
DR0 – Outlet of Lake St. Clair	A, B, B/C	23	0	0%	19	0	0%
DR1 – u/s of Belle Isle	A – C	23	0	0%	19	0	0%
DR2 – Belle Isle	A – C	23	0	0%	19	0	0%
DR3 – Renaissance Center	A – C	23	0	0%	19	0	0%
DR4 – Fort Wayne	A – C	23	0	0%	19	0	0%
DR5 – d/s Rouge River	A, B, B/C	23	1	4%	19	0	0%
DR6 – d/s Ecorse Creek	A – E	23	2	9%	19	0	0%
DR7 – Trenton Channel	A – C	23	2	9%	19	2	9%
DR7 – west side of Grosse Ile	D – G	23	1	4%	19	0	0%
DR8 – u/s of Lake Erie	B – F	23	1	4%	19	0	0%
Totals		230	7	3%	190	2	1%

Figure 5a. Detroit River Daily Geometric Mean Frequency Distribution – Dry Conditions

Figure 5b. Detroit River Daily Geometric Mean Frequency Distribution – Wet Conditions

Figure 6a. Spatial Distribution of Average *E. coli* Concentrations across the Upper Detroit River (cfu/100mL)

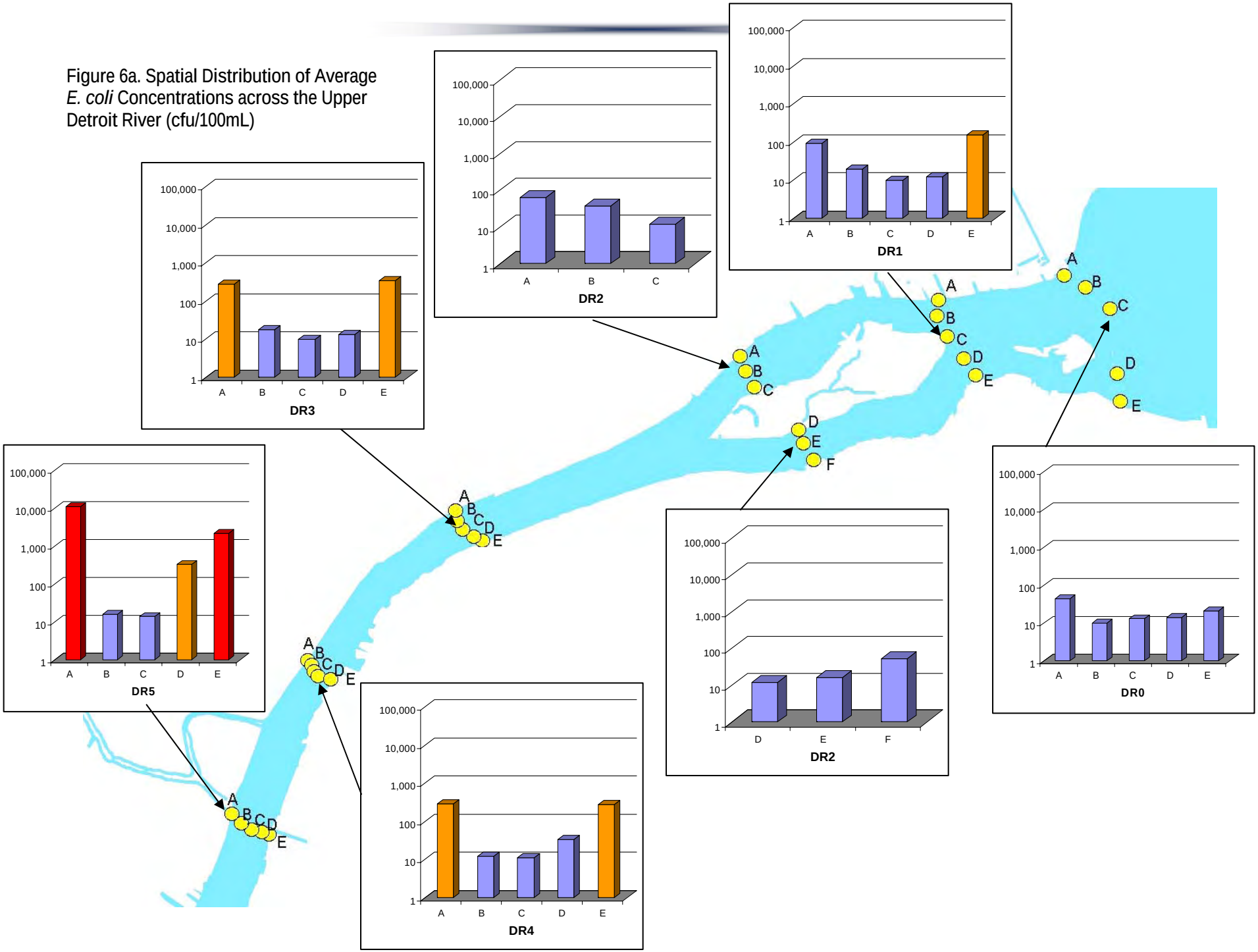
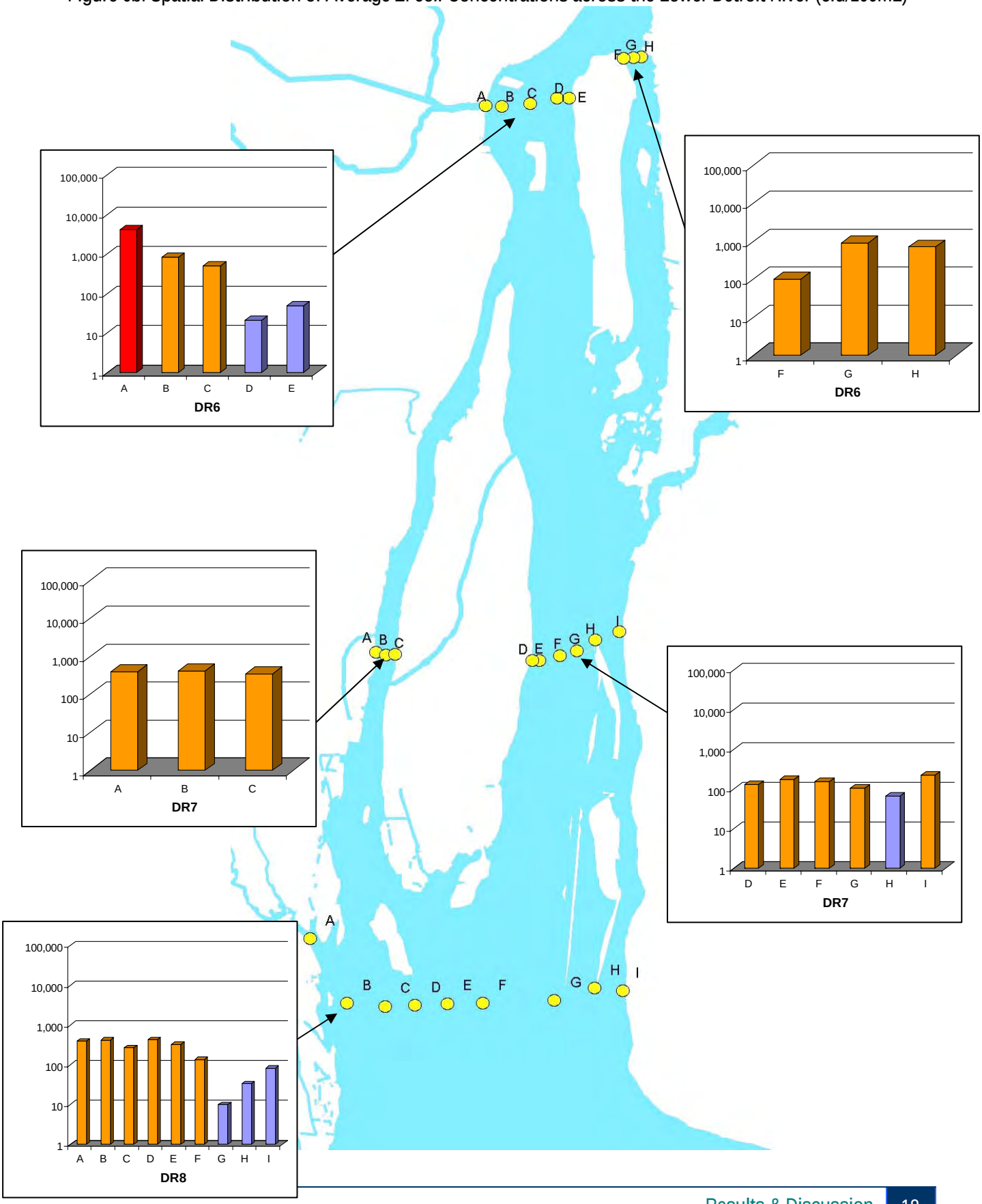


Figure 6b. Spatial Distribution of Average *E. coli* Concentrations across the Lower Detroit River (cfu/100mL)



ECORSE RIVER *E. COLI* CONCENTRATIONS

The *E. coli* concentrations on the North and South Branches of Ecorse Creek were typically very high. The minimum, maximum, median and geometric mean values for each sampling location are shown in Table 7. Based on the average values, the highest *E. coli* values on the North Branch and South Branch were found at Pelham Road (EC2) and Fort Street (EC5) where averages were above 15,000 cfu/100mL.

With elevated *E. coli* concentrations in the open watercourses of the watershed, it was not surprisingly that the enclosed LeBlanc Drain storm sewer concentrations were high as well. Even the minimum *E. coli* values on the LeBlanc Drain were high, with minimum values for three sites above 1,000 cfu/100mL. Furthermore, the highest average concentrations on the Drain exceeded 90,000 cfu/100mL at EC10B and EC8B. As mentioned earlier, a sanitary sewer from a mobile home park was found discharging upstream of EC8B. Sampling stopped at this site when this illicit connection was discovered.

Table 7. Ecorse River Geometric Mean Statistics (sites arranged upstream to downstream)

Sampling Sites	Daily Geometric Means (cfu/100mL)				
	Number of Values	Minimum	Maximum	Median	Average
EC0 – Outlet of Ecorse River	3	2,500*	11,000*	2,900*	5,467*
North Branch Ecorse Creek					
EC4 - Smith Street	17	62	30,300	2,257	4,175
EC3 - Beech Daly Road	23	191	15,715	1,651	4,014
EC2 - Pelham Road	16	22	108,774	5,227	15,329
EC1 - Fort Street	23	136	21,828	1,305	3,639
South Branch Ecorse Creek					
EC7 - Beech Daly Road	23	148	19,480	1,843	3,153
EC6 - Pelham Road	23	17	15,874	2,280	3,204
EC5 - Fort Street	21	1,437	105,762	7,013	16,850
LeBlanc Drain					
EC10A - South pipe at Hartwick and Wayne	16	420*	40,000*	5,050*	10,395*
EC10B - North pipe at Hartwick and Wayne	14	2,200*	590,000*	30,000*	98,671*
EC8A - South pipe at Capitol and Howard	23	1,100*	180,000*	7,600*	22,117*
EC8B - North pipe at Capitol and Howard	8	5,800*	280,000*	70,000*	93,863*
EC9 - Drain outlet	9	700*	70,000*	7,000*	14,689*

*Based on single analytical values, not daily geometric means

As with the Detroit River data, the Ecorse Creek daily geometric mean values were evaluated based on weather conditions. The resulting daily geometric means are displayed on box plots that were developed for the North Branch, South Branch, and the LeBlanc Drain. This analysis reveals an unusual phenomenon at some of the monitoring locations. Dry weather values are generally the same as the wet weather values at sites EC2, EC5, EC8 and EC10. In most urban watersheds in southeast Michigan, dry weather values are much lower than wet weather values. This indicates the presence of dry weather sources upstream of these locations (See Figures 7a, 7b and 7c).

How to Interpret a Box and Whiskers Plot

*The **median value** is the center point of the data set, regardless of the values. 50% of the data are above this value, and 50% of the data are below it.*

*The **box** portion of the plot contains a total of 50% of the data. The box incorporates 25% of data directly above the median value and the 25% of data directly below the median value. The top of the box signifies the 75th **percentile** (75% of the data is below this point). The bottom of the box signifies the 25th **percentile** (25% of the data lies below this point). The larger the box, the more variable the data.*

*The **whiskers** encapsulate 80% of the data. The top whisker represents the 90th percentile, and the bottom whisker represents the 10th percentile.*

*Any data that does not fall within these values, are considered **outliers**, and are displayed as dots outside of the whiskers.*

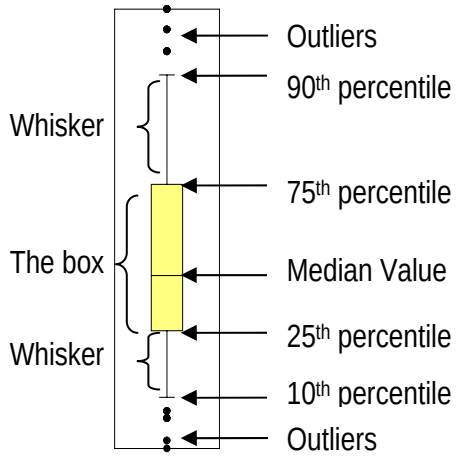


Figure 7a. North Branch Ecorse Creek Daily Geometric Means

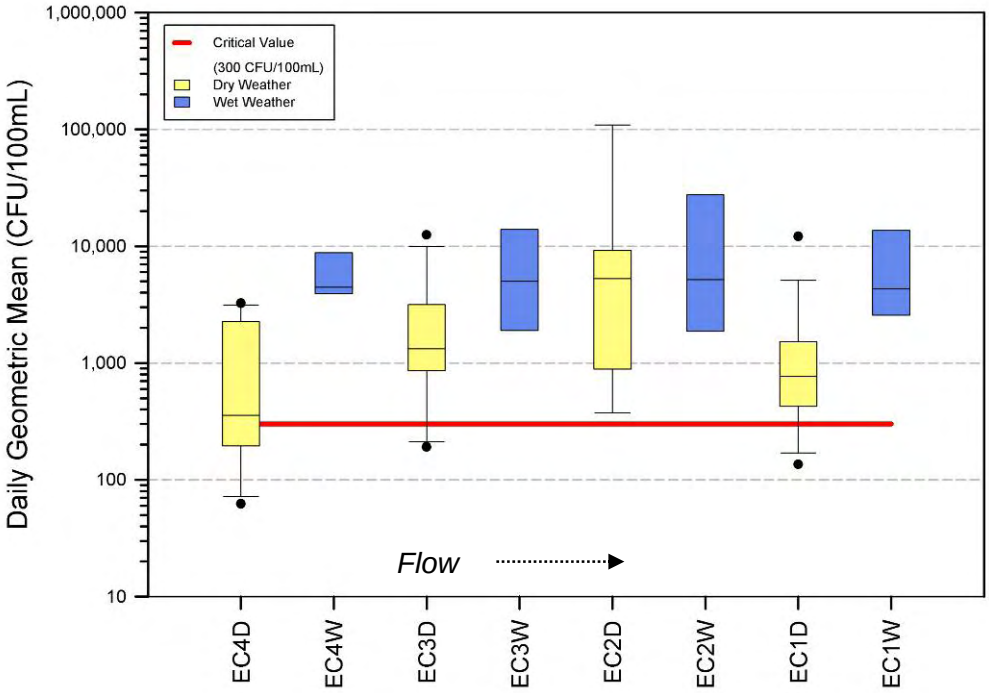


Figure 7b. South Branch Ecorse Creek Daily Geometric Means

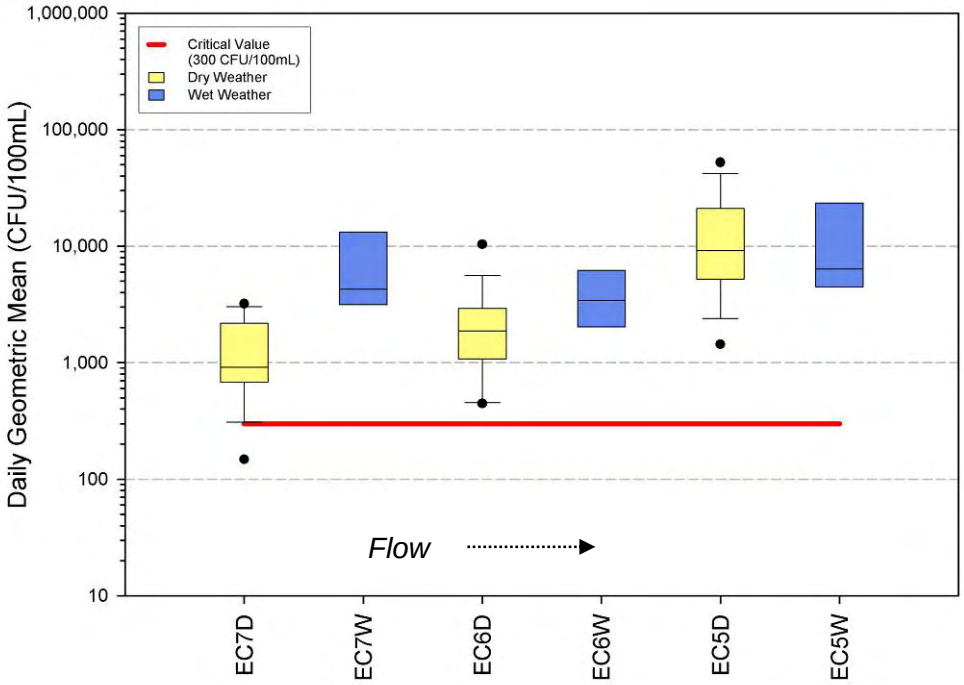
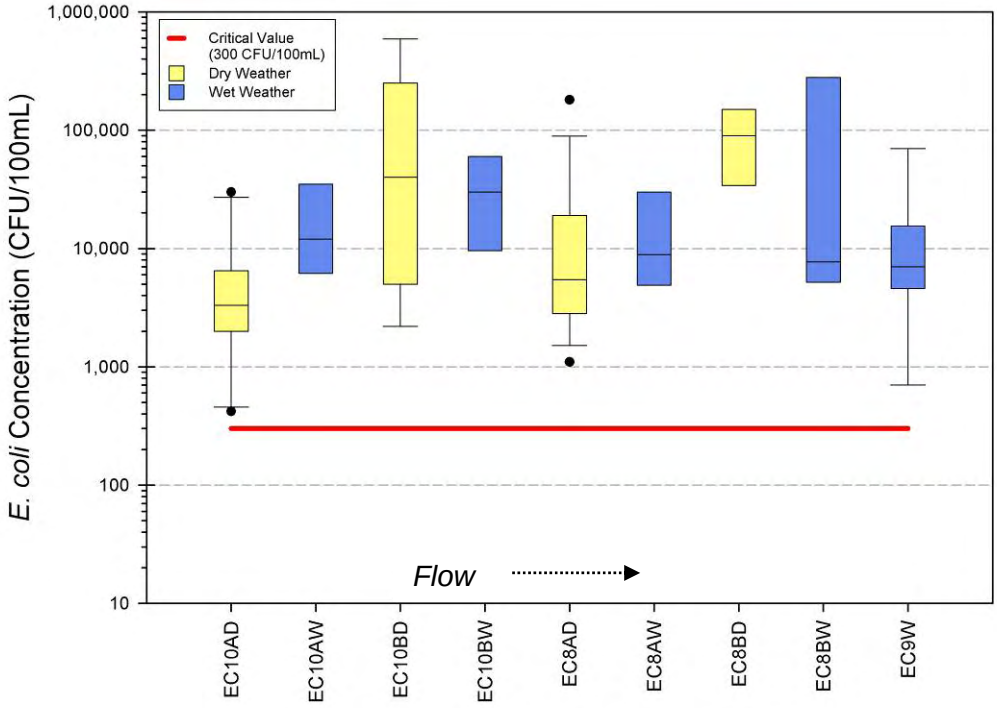


Figure 7c. LeBlanc Drain *E. coli* Concentrations



The median values of the wet weather samples are fairly constant on the North Branch at around 4,000 to 5,000 cfu/100mL. The median dry weather values seemed to peak at the Pelham Road site (EC2) at approximately 5,000 cfu/100mL. The dry weather *E. coli* concentrations appear to drop at the next downstream site, Fort Street (EC1), where the median value was around 800 cfu/100mL. On the South Branch, the wet weather median values were in the same range as the North Branch, where they varied between 3,000 and 6,500 cfu/100mL. The highest median dry and wet weather values were at the most downstream location, Fort Street (EC5).

Analysis of the Ecorse River data revealed that both the North and South Branch of Ecorse Creek rarely met WQSs. On a site by site basis, 76% to 100% of the daily geometric means exceeded the full body contact standard of 300 cfu/100mL and 52% to 100% of the geometric means exceeded Michigan's partial body contact standard of 1,000 cfu/100mL. Further emphasis of the water quality problems are shown in the 30-day geometric mean comparisons, where all of the values exceeded the 30-day standard of 130 cfu/100mL. Table 8 depicts the comparisons to WQSs, while Figures 8a and 8b depict the distribution of the geometric mean data graphically.

Table 8. Ecorse River Water Quality Exceedences

Sampling Sites	Daily Geometric Means			30-Day Geometric Means		
	No. of Values	Exceedences (>300 cfu/100mL)		No. of Values	Exceedences (>130 cfu/100mL)	
		Number	Percent		Number	Percent
North Branch Ecorse Creek						
EC4 - Smith Street	17	13	76%	6	6	100%
EC3 - Beech Daly Road	23	21	91%	19	19	100%
EC2 - Pelham Road	16	15	94%	2	2	100%
EC1 - Fort Street	23	21	91%	19	19	100%
South Branch Ecorse Creek						
EC7 - Beech Daly Road	23	22	96%	19	19	100%
EC6 - Pelham Road	23	22	96%	19	19	100%
EC5 - Fort Street	21	21	100%	12	12	100%
Totals	146	135	92%	86	86	100%

The LeBlanc Drain had higher *E. coli* concentrations than found in the open branches of Ecorse Creek. In fact, ninety-five percent of the samples exceeded 1,000 cfu/100mL and forty-one percent of the samples exceeded 10,000 cfu/100mL (See Table 9).

Table 9. LeBlanc Drain *E. coli* Concentration Distribution

Sampling Sites	Total No. of Values	*E. coli* Range (cfu/100mL)			
		0 - 300	301 – 1,000	1,001 – 10,000	> 10,000
EC10A - South pipe at Hartwick and Wayne	16	0	2	9	5
EC10B - North pipe at Hartwick and Wayne	14	0	0	6	8
EC8A - South pipe at Capitol and Howard	23	0	0	14	9
EC8B - North pipe at Capitol and Howard	8	0	0	3	5
EC9 - Drain outlet	9	0	1	6	2
Totals	70	0	3 (4%)	38 (54%)	29 (41%)

Figure 8a. Ecorse River Daily Geometric Mean Frequency Distribution – Dry Conditions

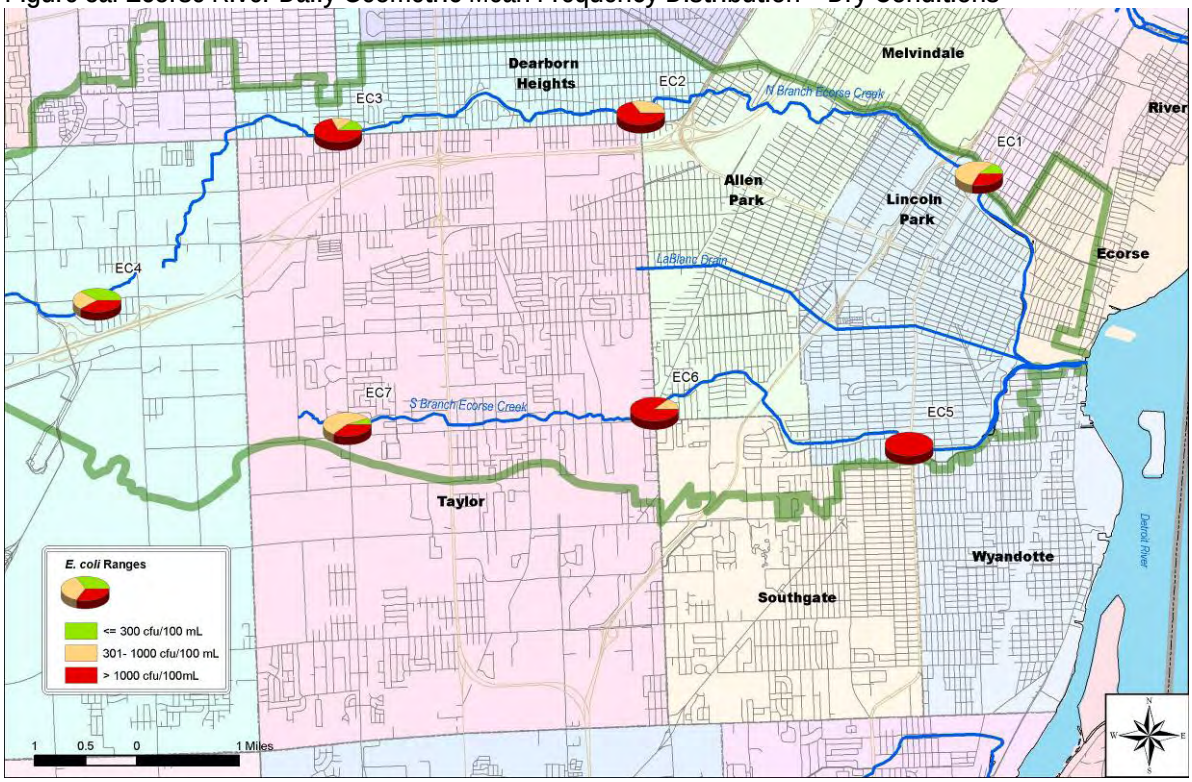
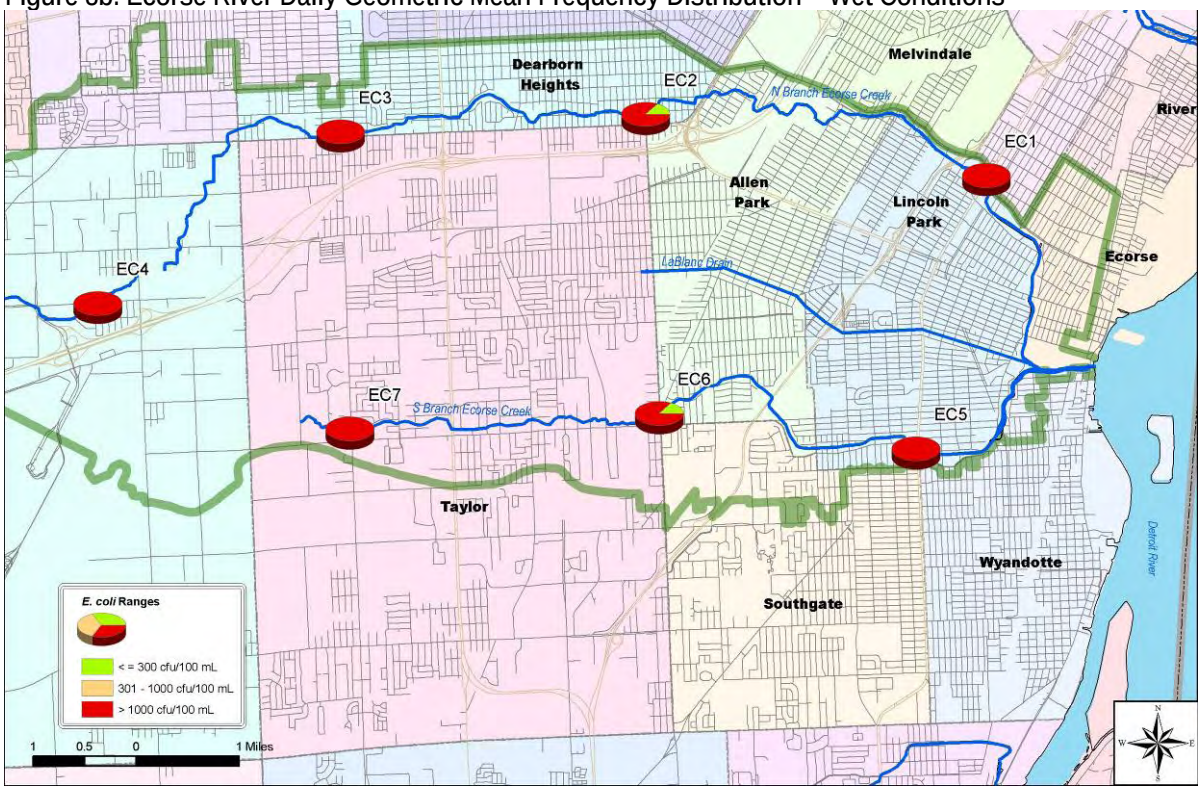


Figure 8b. Ecorse River Daily Geometric Mean Frequency Distribution – Wet Conditions



BACTERIAL SOURCE TRACKING RESULTS

It should be emphasized that bacterial source testing was only used on a limited number of samples at a select number of locations. Since comprehensive testing was not performed (multiple samples over a long period of time), a "negative" result at any given site doesn't mean that human contamination is not present at that site, only that it was not present in that particular sample.

Detroit River

Four bacterial source tracking (BST) samples were collected on the Detroit River. As mentioned earlier, the selection of the BST sites were based on the *E. coli* results for each site from earlier sampling events. Also noted earlier, due to budget constraints most of the BST sampling came at the end of the monitoring period when additional funding was allotted by the EPA.

Up to August 21, 2007, the highest *E. coli* concentrations on the Detroit River were found near the Canadian shoreline at locations DR3E, DR4E, DR5E and DR6G. These high values were associated with wet weather conditions. Therefore, these sites were subject to BST analysis during wet weather conditions.

Results from two of the four sites, DR3E (Renaissance Center) and DR4E (Fort Wayne), showed presence of the human biomarker using the Human *Enterococcus* ID™ indicating the presence of *E. coli* from human sources (See Figure 9a).

Ecorse River

Due to the widespread *E. coli* problems in the Ecorse River watershed, all of the sites were selected for BST analysis during both dry and wet conditions, except the outlet of the LeBlanc Drain (EC9), the outlet of Ecorse River (EC0) and on the LeBlanc Drain at EC8B (a known human source was already found here). Generally, each site was sampled once during dry and once during wet conditions.

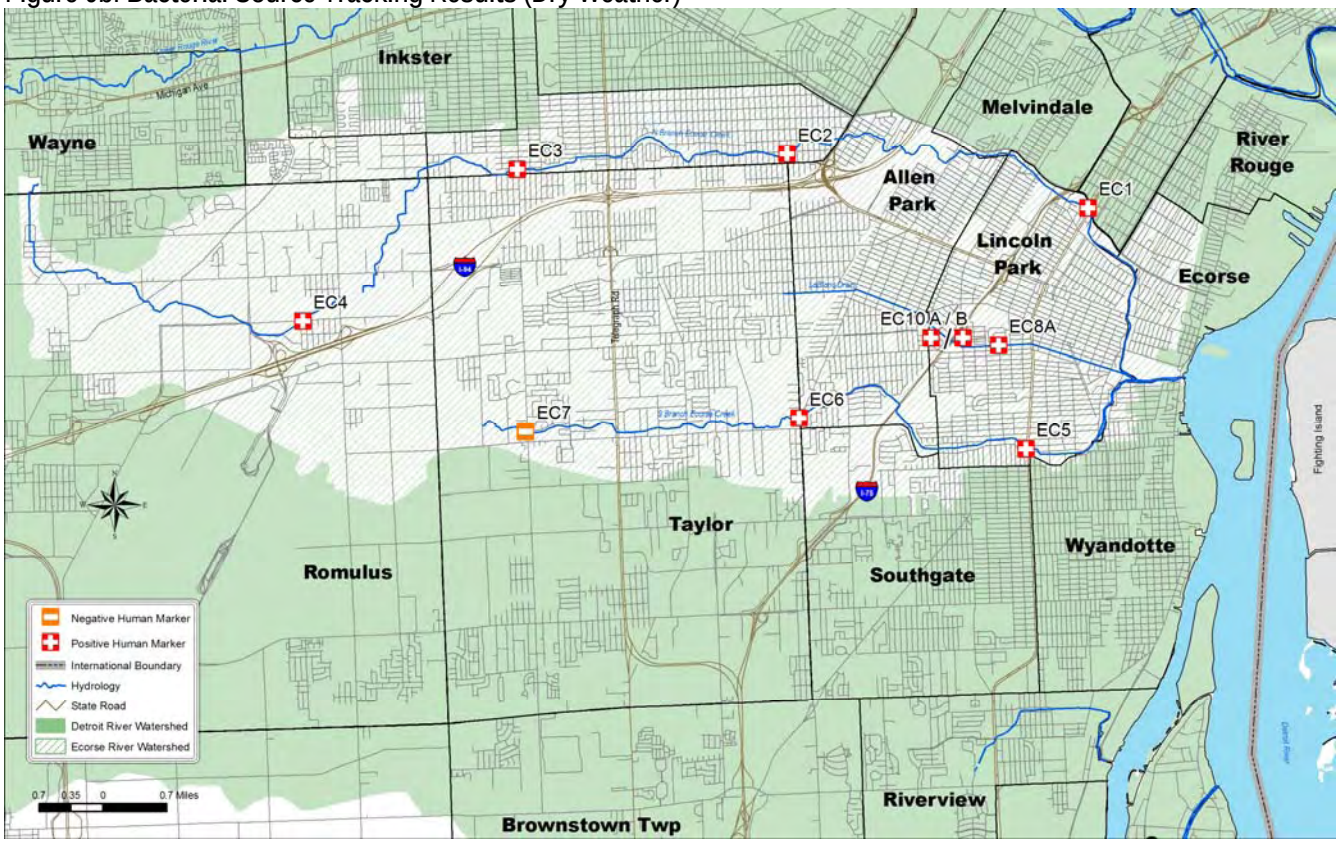
During dry conditions, the human biomarker was present at all sites on the North and South Branches and on LeBlanc Drain, except EC7 (South Branch at Beech Daly). To confirm the negative result initially found at EC7, the site was sampled two more times for BST analysis. The results were always negative for the human biomarker, giving a strong indication that *E. coli* from human sources was not impacting this site during dry conditions (See Figure 9b).

During wet conditions, fewer positive results were found in the watershed. The human biomarker was found at sites EC7, EC8A and EC10B indicating the presence of *E. coli* from human sources at these sites (See Figure 9a). Based on experience in other watersheds and the dry weather conditions, it is likely that positive results would have been found at all sites if repeated sampling could have been performed during wet conditions.

All project BST data, the associated *E. coli* and *Enterococcus* concentrations are reported in Appendix E, by weather condition.

Figure 9a. Bacterial Source Tracking Results (Wet Weather)

Figure 9b. Bacterial Source Tracking Results (Dry Weather)



Quality Control Review

All project efforts from sample collection to laboratory analysis were performed under the guidance of a Quality Assurance Project Plan (QAPP), which was approved by the MDEQ and EPA prior to the start of sampling. An analysis of the data precision and completeness is provided below.

PRECISION

Field precision, or the ability of the sampling team to collect two samples with a high degree of similarity, was assessed by the collection and submission for analysis of field duplicate samples. Field duplicate samples were collected from the same location, at the same time, using the same sampling method, and were then independently analyzed in the same manner. One duplicate sample was collected for every ten *E. coli* samples.

For quantitative microbiological analysis, precision is calculated using the method outlined in Standard Methods for the Examination of Wastewater, 18th Edition, section 9020B.4.b:

$$RPD_{bacteria} = (\log X_1 - \log X_2)$$

The $RPD_{bacteria}$ should be lower than $3.27\sum R_{log} / n$, where R_{log} is the difference in the natural log of replicate samples for the first or most recent set of 15 samples, and where n is the number of samples.

All but one of the reviewed duplicate sample sets fell within the acceptable range for precision as described above. The ten analytical results closest to the one out of range duplicate were flagged (See Appendices C & D).

ACCURACY

Accuracy was assessed by the laboratory staff through the analysis of positive and negative controls. Negative controls were also used by field staff in the form of blank samples. One blank sample was collected for every twenty *E. coli* samples throughout the course of the field effort. All blanks were handled and analyzed in the same manner as the river samples. Three of the eighty-six blanks were found to be at or above the sample detection limit of 10 cfu/100mL. The twenty analytical results closest to the three out of range blanks were flagged (See Appendices C & D).

COMPLETENESS

Every effort was made to obtain valid data for each sampling point at all times. Completeness was measured by dividing the number of usable sample results by the total number of sample results. The completeness objective for this project was 90%.

As mentioned above, one of the duplicate samples collected from Ecorse Creek did not meet the precision requirements. The laboratory and field crew reviewed their procedures and found no erroneous behavior, thus it was the project QA/QC manager's opinion that the analytical results from the 10 samples collected closest to the erroneous duplicate sample were kept and used in calculations, but flagged.

Also noted earlier, three blank results were above the detection limit. These blanks were taken during sampling events 2 and 3. Following this discovery, the field manager reviewed sample collection procedures with the field staff. This review included how to handle the laboratory bottles and a reminder to wear clean latex gloves at each sampling site. No further problems occurred following this discussion.

The analytical results surrounding two of the out of range blanks were below the detection limit and the results surrounding the third blank were much higher than the detection limit, but consistent with other site results. Based on this information, it was the opinion of the project QA/QC manager that the problem was isolated in nature and not systemic. Thus, the twenty analytical results surrounding the faulty blanks were flagged, but used in project calculations.

This resulted in a total project completeness factor of 96%. Thus, the project completeness objective was met (See Table 10).

Table 10. Completeness Calculations

	No. of samples collected	No. of samples flagged	No. of usable samples	% Complete
Detroit River	1,311	40	1,271	97%
Ecorse Creek	511	30	481	94%
Total	1,822	70	1,752	96%

DEVIATIONS FROM THE QAPP

The original QAPP was approved on May 3, 2007. The first sampling event was conducted with verbal approval of the QAPP, but not written approval. Since the sampling procedures and analytical methods utilized during the first event were consistent with the QAPP and all subsequent effort, the data was judged valid and kept for final data analysis.

The QAPP was revised twice throughout the course of the project: once on August 6th and August 20th. These modifications were necessary to account for the change in *E. coli* sampling sites along Ecorse Creek and to account for the BST sampling locations, which were determined as the project proceeded.

No other deviations to the QAPP occurred throughout the course of the project. The final approved QAPP dated August 20, 2007 is included in Appendix B.

Conclusions

DETROIT RIVER

There were infrequent exceedances of water quality standards on the Detroit River as indicated by *E. coli* concentrations. Michigan's daily WQS for *E. coli* (300 cfu/100mL) was exceeded in three percent of the calculated geometric means for the US portion of the River. Based on the 2007 monitoring data, elevated *E. coli* concentrations are associated with wet weather conditions and are generally restricted to the US and Canadian near shore zones (within 50 feet of the shoreline). The highest *E. coli* concentrations were found at the confluence of the Rouge and Ecorse rivers in Michigan and upstream of Turkey Creek in Ontario.

Limited bacterial source tracking analysis was conducted on the Detroit River. This analysis revealed that *E. coli* from a human source(s) was present in two of four samples from the River upstream of the Rouge River near the Canadian shoreline. These samples were collected during wet weather conditions indicating that contamination was likely entering the River via combined sewer overflows and/or sanitary sewer overflows.

ECORSE RIVER

There were frequent, almost consistent, exceedances of water quality standards on the Ecorse River, as indicated by *E. coli* concentrations. Collectively the North Branch and South Branches of Ecorse Creek exceeded Michigan's WQSs for the daily and 30-day standard 92 and 100% of the time, respectively. In fact, 73% of the daily geometric means were above the partial body contact standard of 1,000 cfu/100mL.

The LeBlanc Drain had higher *E. coli* concentrations than found in the open branches of Ecorse Creek. On the LeBlanc Drain ninety-five percent of the samples were above 1,000 cfu/100mL and forty-one percent of the samples were above 10,000 cfu/100mL. These elevated E. coli levels were associated with both dry and wet weather conditions.

E. coli from human sources was found in the North and South Branches or Ecorse Creek and in the LeBlanc Drain, as indicated by the BST results. The likely sources include illicit connections from sanitary sewers in dry weather, as well as contaminated stormwater runoff and sanitary sewer overflows in wet weather.

References

ECIC 2006. *Ecorse Creek Watershed Management Plan*. Ecorse Creek Inter-Municipality Committee. Online at: **http://www.allianceofdownriverwatersheds.com/initiatives/**

Hamdy, Y., Klemens, D. Oliver, B., et al. *Upper Great Lakes Connecting Channels Study Final Report Volume II.* December 1988.

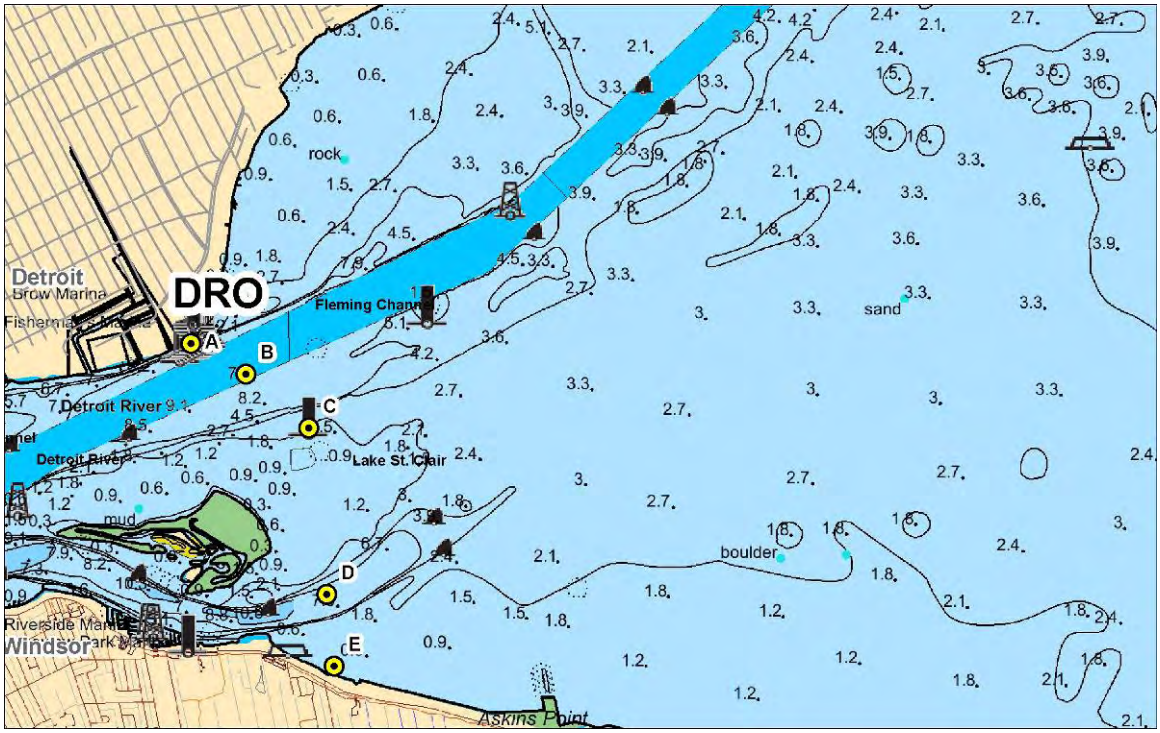
Holtschlag, David J., John A. Koschik. "*Steady-State Flow Distribution and Monthly Flow Duration in Selected Branches of St. Clair and Detroit Rivers within the Great Lakes Waterway*" Water-Resources Investigations Report 01-4135. USGS: Lansing, MI. August, 2001.

Appendix A
Monitoring Site Descriptions

Detroit River Transects and Sampling Sites

DR0. Detroit River at Windmill Pointe Park and upstream of Peche Island (5 samples; A - E).

	Latitude	Longitude
A)	42.35835064	-82.92703645
B)	42.35633224	-82.92241775
C)	42.35288515	-82.91712634
D)	42.34237053	-82.91586434
E)	42.33782121	-82.91531816

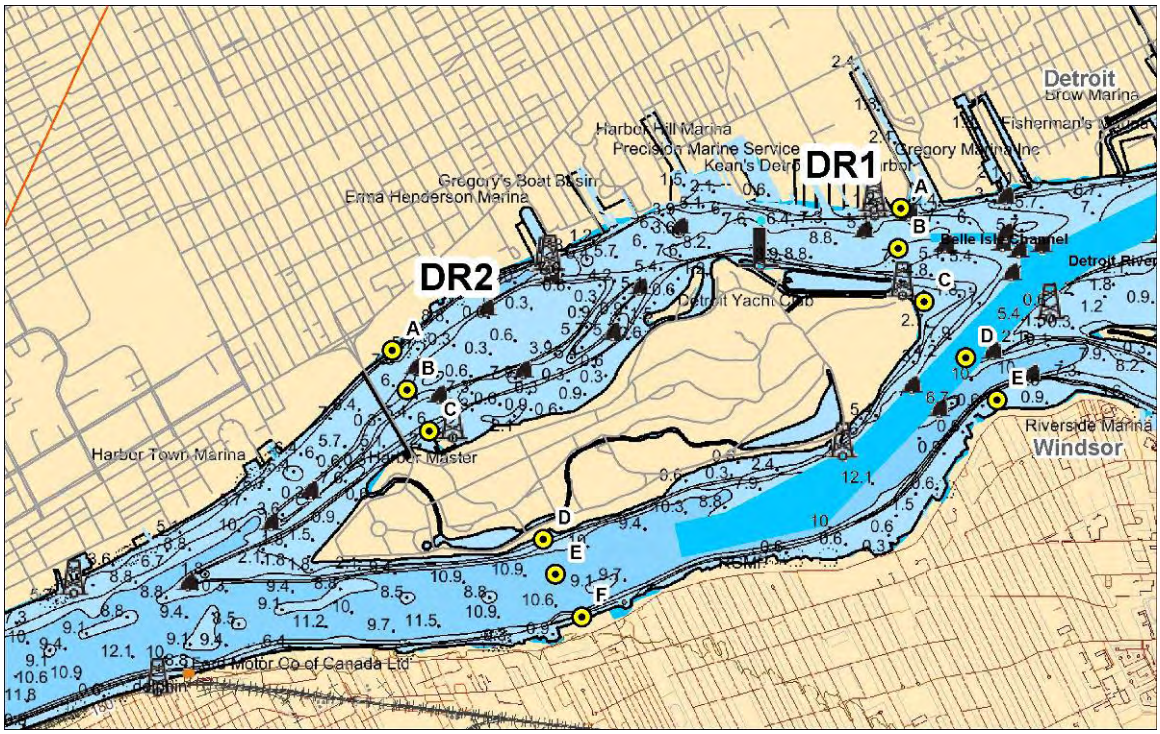


DR1. Detroit River just downstream of Conner Creek and upstream of Belle Isle (5 samples; A - E).

	Latitude	Longitude
A)	42.35468108	-82.95453615
B)	42.35214406	-82.95480891
C)	42.34877209	-82.95269410
D)	42.34518328	-82.94919412
E)	42.34250257	-82.94662352

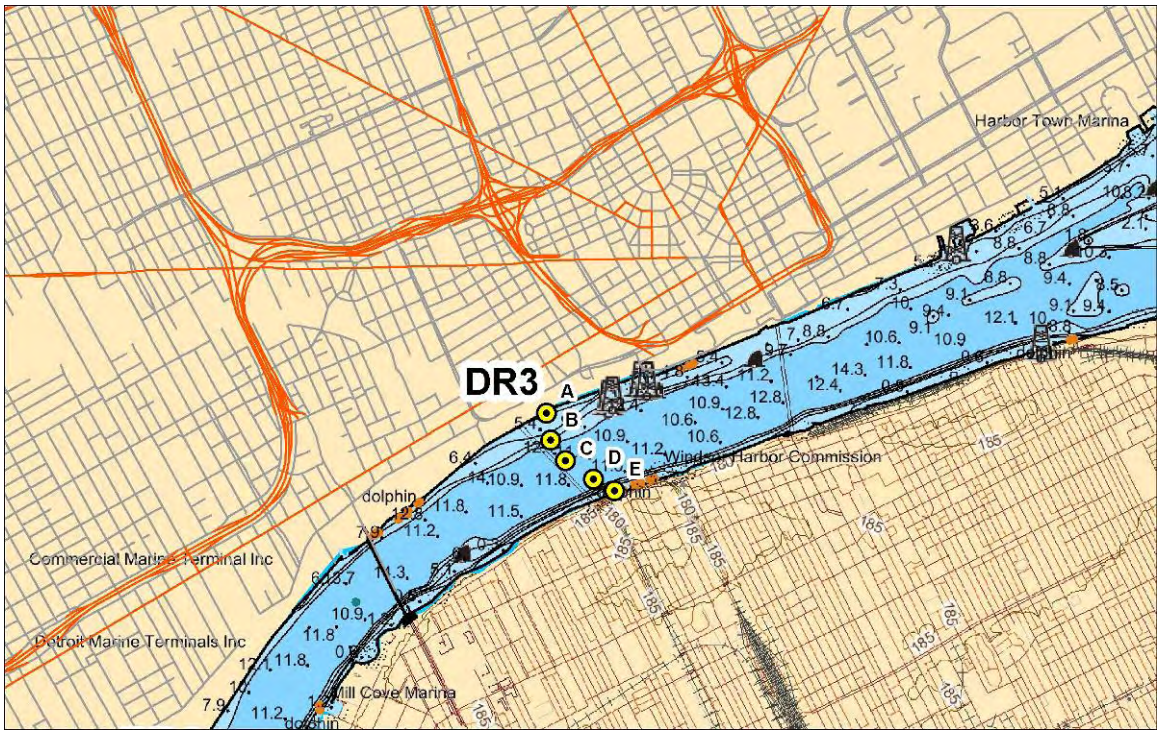
DR2. Scott Middle Ground near Detroit Boat Club – (3 samples; A - C); and Fleming Channel – south side of Belle Isle- (3 samples; D - F).

	Latitude	Longitude
A)	42.34624230	-82.99793904
B)	42.34374190	-82.99675600
C)	42.34112433	-82.99492316
D)	42.33418801	-82.98538315
E)	42.33195896	-82.98439809
F)	42.32923393	-82.98222791



DR3. Detroit River downstream of Renaissance Center (5 samples; A - E).

	Latitude	Longitude
A)	42.32199938	-83.06036382
B)	42.32031604	-83.06003891
C)	42.31899553	-83.05882954
D)	42.31781006	-83.05646123
E)	42.31704121	-83.05468874

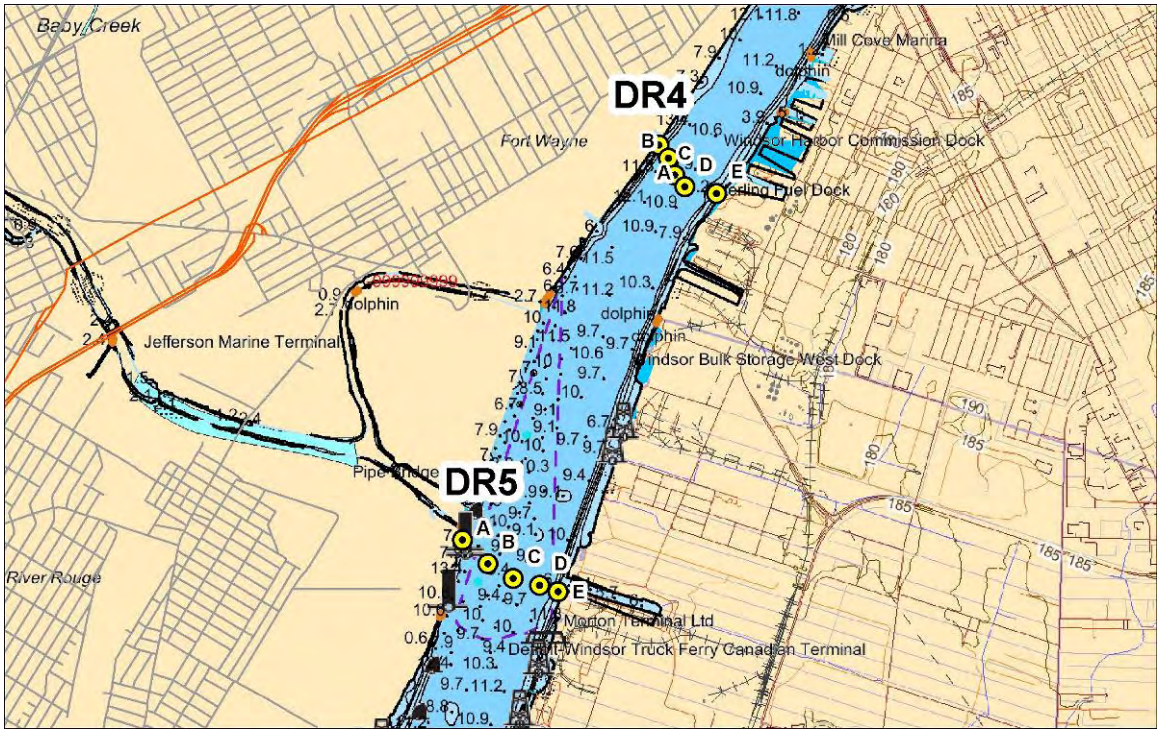


DR4. Detroit River at Fort Wayne (5 samples; A – E.)

Latitude	Longitude
A) 42.29815947	-83.09301218
B) 42.29733558	-83.09218527
C) 42.29625858	-83.09167842
D) 42.29550675	-83.09083794
E) 42.29504390	-83.08816116

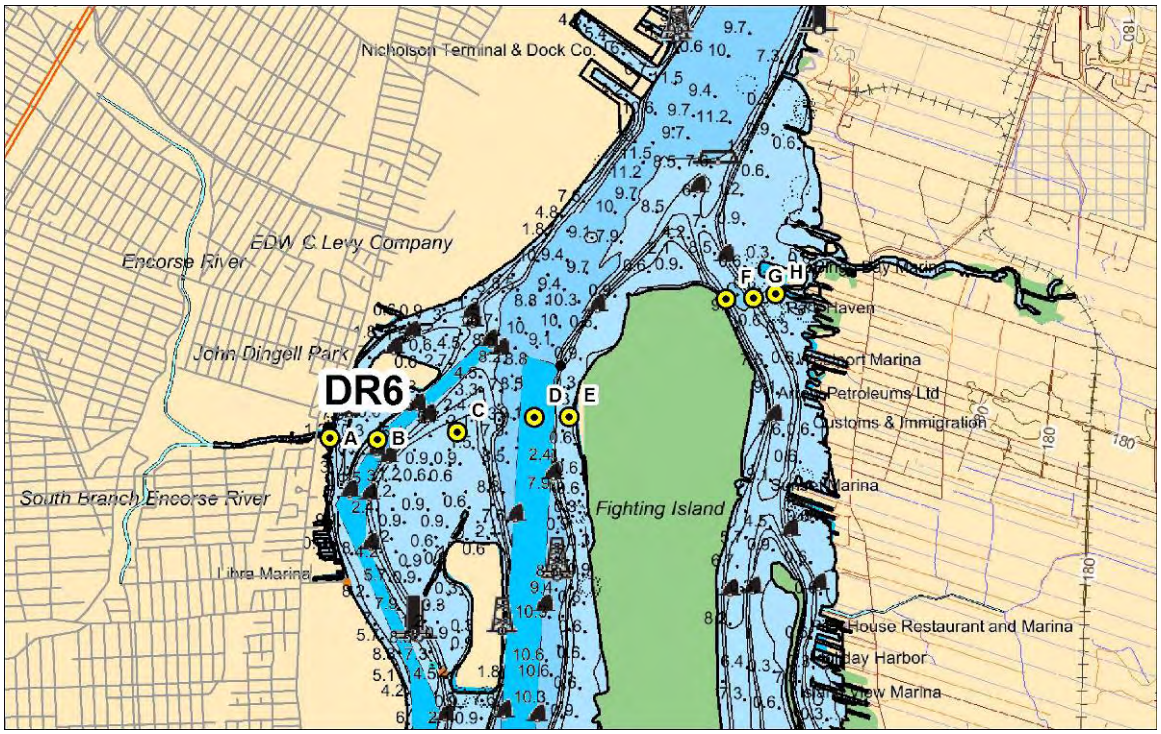
DR5. Detroit River downstream of Zug Island at Rouge Edison Power Plant (left bank sample within 50' of large steel circular mooring cribs) (5 samples; A - E).

Latitude	Longitude
A) 42.27346185	-83.11016713
B) 42.27194270	-83.10808243
C) 42.27096137	-83.10591027
D) 42.27050145	-83.10368295
E) 42.27010370	-83.10211272



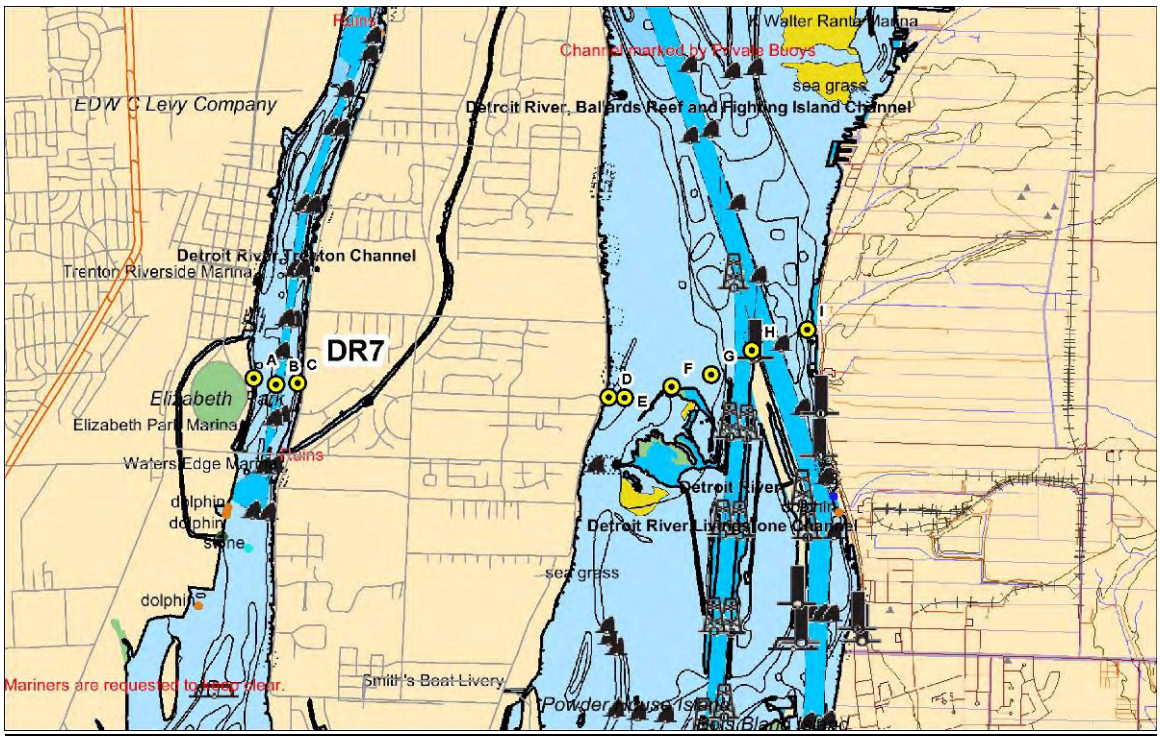
DR6. Detroit River downstream of Ecorse River (5 samples; A - E); and Detroit River between the northern end of Fighting Island and Canadian mainland, upstream of Canadian Grassy Island (3 samples; F - H).

	Latitude	Longitude
A)	42.23474107	-83.14734236
B)	42.23458321	-83.14332364
C)	42.23500221	-83.13652997
D)	42.23586082	-83.12995574
E)	42.23584141	-83.12699253
F)	42.24314941	-83.11353633
G)	42.24319161	-83.11121634
H)	42.24341662	-83.10933156



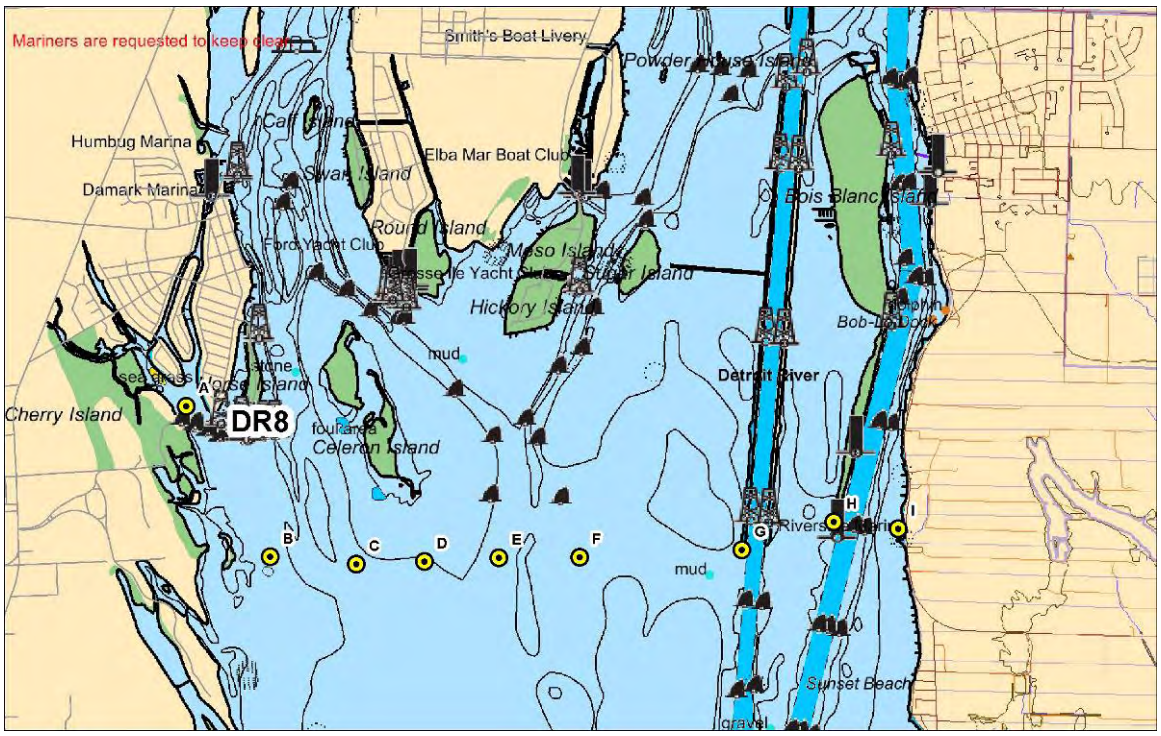
DR7. Trenton Channel near Elizabeth Park (3 samples; A - C); and Livingstone Channel upstream of Stoney Island between Grosse Ile and the Canadian mainland. (6 samples; D - I).

Latitude	Longitude
A) 42.13333648	-83.17582879
B) 42.13281282	-83.17350286
C) 42.13291022	-83.17120609
D) 42.13140088	-83.13804217
E) 42.13136715	-83.13636504
F) 42.13219583	-83.13134014
G) 42.13309550	-83.12708265
H) 42.13498553	-83.11678123
I) 42.06743985	-83.17496398



DR8. Detroit River downstream of the confluence of Brownstown Creek and the Frank & Poet Drain (1 sample: A); and Detroit River downstream of Celeron Island across the shipping channels to the Canadian Mainland. (8 samples: B – I)

Latitude	Longitude
A) 42.08017189	-83.19282900
B) 42.06814735	-83.18409027
C) 42.06743985	-83.17496398
D) 42.06756631	-83.16772411
E) 42.06778535	-83.15976708
F) 42.06773695	-83.15117024
G) 42.06812587	-83.13384394
H) 42.07023456	-83.12402730
I) 42.06965448	-83.11718788

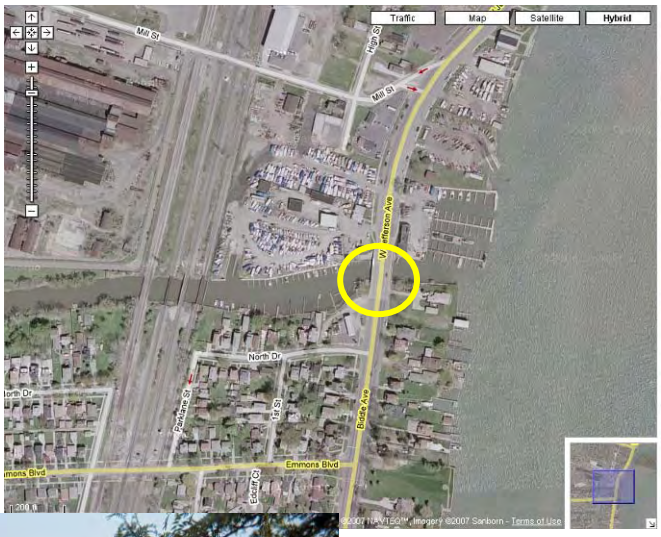


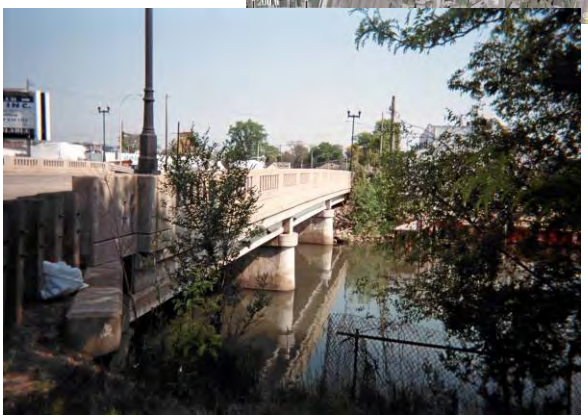
Ecorse Creek Monitoring Sites

EC0. Ecorse River @ West Jefferson Ave. (only during wet conditions, if flow is present)
Sample A.

NOTE: It was determined that the boat sample for DR6A is collected within 50 feet of the EC0 location, and therefore sampling at EC0 was abandoned.

Latitude	Longitude
42.23496565	-83.14826818





EC1. Ecorse Creek @ Fort Street, just south of Fort/Outer Drive intersection.
Samples A – C.

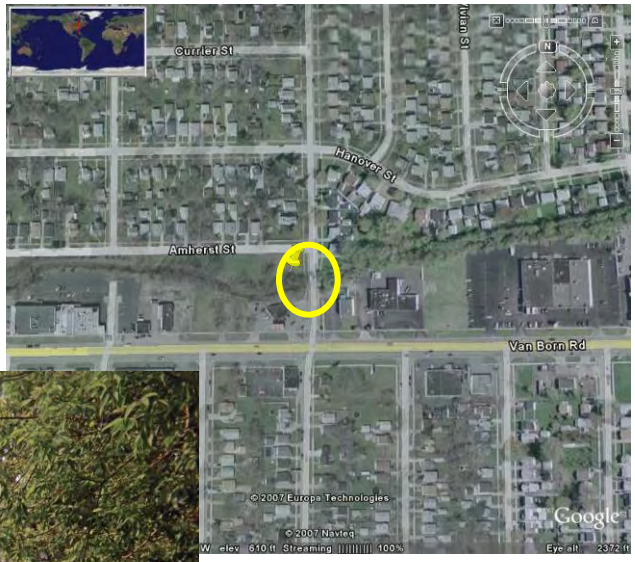
Latitude	Longitude
42.26204142	-83.16762035

EC2. Ecorse Creek @ Pelham Rd, just north of Van Born Rd.
Samples A – C.

Latitude	Longitude
42.27131387	-83.23193383

EC3. Ecorse Creek @ Beech Daly, south of Amherst St., west of Beech Daly. Same location as USGS gauging station #04168580.
Samples A – C.

Latitude	Longitude
42.26941335	-83.28970221

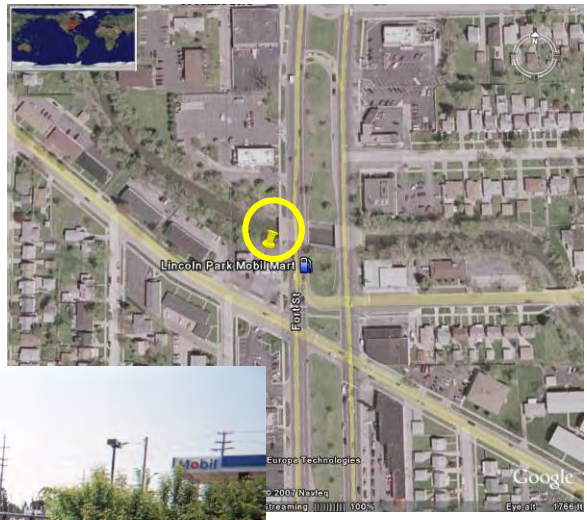


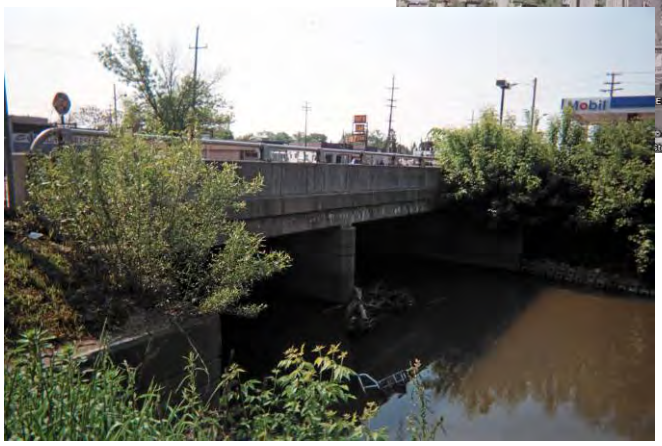
EC4. Ecorse Creek @ Smith Rd; located between Middlebelt and Merriman on Smith. Just West of Kenwood St. Samples A – C.

Latitude	Longitude
42.2457074	-83.3360464

EC5. South Ecorse Creek @ Fort Street, just north of Fort/Goddard Rd intersection. Access from gas station at NW corner of intersection.
Samples A – C.

Latitude	Longitude
42.22375458	-83.18165691





EC6. South Ecorse Creek @ Pelham Rd, located just north of Pelham/Goddard Rd intersection. Access from vacant gas station on west side of Pelham.
Samples A – C.

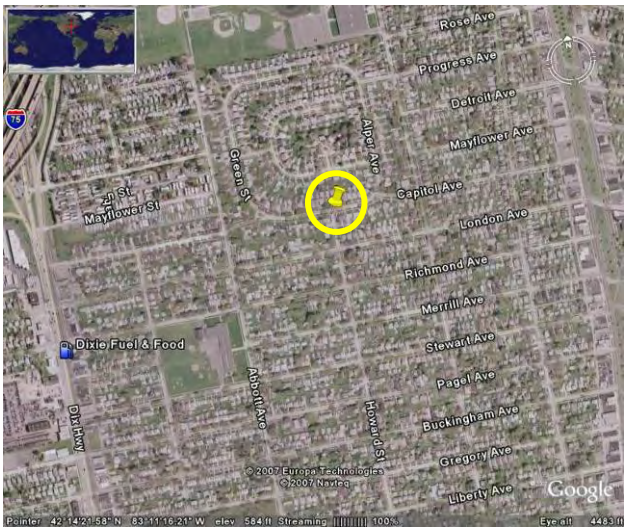
Latitude	Longitude
42.22921665	-83.23010768

EC7. South Ecorse Creek @ Beech Daly, located north of Beech Daly/Goddard intersection. Access from east side of road.
Samples A – C.

Latitude	Longitude
42.22760215	-83.28865524

LeBlanc Drain

EC8. Capital Rd. & Howard, three blocks west of Fort St on Capitol. Capital is between Goddard and Champaign on Fort. Two manholes; one in the center of the road and the other on the south edge of the road. Sample A-B.



NOTE: Sampling was discontinued on EC8B after June 19, 2007 an illicit connection was discovered just upstream of this sampling point. EC10 was added at this point.

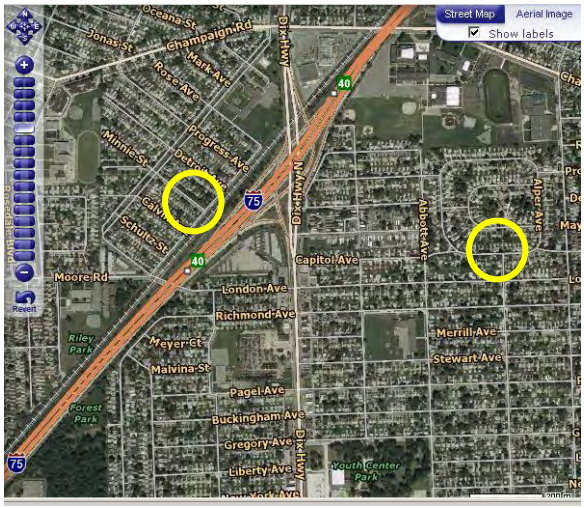
Latitude	Longitude
42.24035636	-83.18718233

EC9. LeBlanc Drain outlet at Council Point Park, east of red, white and blue monuments (only during wet conditions, if flow is present). Sample A

Latitude	Longitude
42.23544598	-83.15983538

EC10. Hartwick Rd. & N Wayne St. Hartwick is between Goddard and Champaign. Two manholes; both are in the road. Sample A-B.

Latitude	Longitude
42.2416850	-83.198242



Appendix B
Quality Assurance Project Plan

Pathogen Sampling for the Detroit River and Tributaries in support of Total Maximum Daily Load Development

Quality Assurance Project Plan

Task Order No. 039

Prepared for:

U.S. Environmental Protection Agency Region 5
77 West Jackson Boulevard
Chicago, IL 60604-3507

Prepared by:

RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

USEPA Contract Number 68-C-02-110

August 20, 2007
Revision 2

This quality assurance project plan (QAPP) has been prepared according to guidance provided in *EPA Requirements for Quality Assurance Project Plans* (EPA QA/R-5, EPA/240/B-01/003, U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC, March 2001) and *EPA Guidance for Quality Assurance Project Plans for Modeling* (EPA QA/G-5M, EPA/240/R-02/007, U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC, December 2002) to ensure that environmental and related data collected, compiled, and/or generated for this project are complete, accurate, and of the type, quantity, and quality required for their intended use. RTI, International will conduct work in conformance with the quality assurance program described in the procedures detailed in this QAPP.

Approvals:

Julianne Socha EPA Task Order Manager U.S. EPA Region 5	08/20/07 Date	Brenda Sayles Chief – Lake Michigan Unit Assessment Section, Water Bureau MDEQ	8/20/07 Date
Dr. Bill Cooter RTI Task Order Director RTI	Date		
Mark Mikesell ECT QA Officer ECT	Date	Dr. Sanjiv Sinha ECT Project Director ECT	Date
Angella Breitenbeck Laboratory Manager Paragon Labs	Date	Thierry Sam Tamers Laboratory Director Source Molecular Corporation	Date

This quality assurance project plan (QAPP) has been prepared according to guidance provided in *EPA Requirements for Quality Assurance Project Plans* (EPA QA/R-5, EPA/240/B-01/003, U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC, March 2001) and *EPA Guidance for Quality Assurance Project Plans for Modeling* (EPA QA/G-5M, EPA/240/R-02/007, U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC, December 2002) to ensure that environmental and related data collected, compiled, and/or generated for this project are complete, accurate, and of the type, quantity, and quality required for their intended use. RTI, International will conduct work in conformance with the quality assurance program described in the procedures detailed in this QAPP.

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Angella Breitenbeck Laboratory Manager Paragon Labs	Date	Thierry Sam Tamers Laboratory Director Source Molecular Corporation	08-20-07 Date

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Angella Breitenbeck Laboratory Manager Paragon Labs	8-2007 Date	Thierry Sam Tamers Laboratory Director Source Molecular Corporation	Date

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Sample Site Locations

APPENDIX B

Environmental Consulting & Technology Standard Operating Procedures

APPENDIX C

Paragon Laboratory Quality Assurance Documents

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Source Molecular Corporation Quality Assurance Documents

APPENDIX E

DNA Sample Site Locations

ACRONYMS AND ABBREVIATIONS

AWRL	Ambient Water Reporting Limits
CFU	Colony Forming Unit
CSO	Combined Sewer Overflow
DI	Deionized (water)
DNA	Deoxyribonucleic Acid
DQO	Data Quality Objective
E. coli	*Escherichia coli*
ECT	Environmental Consulting & Technology, Inc.
EPA	United States Environmental Protection Agency
GIS	Geographic Information System
GPS	Global Positioning System
MDL	Method Detection Limit
mL	Milliliter
MDEQ	Michigan Department of Environmental Quality
NPDES	National Pollutant Discharge Elimination System
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RPD	Relative Percent Differential
RTI	RTI International
SMC	Source Molecular Corporation
SOW	Scope of Work
SSO	Sanitary Sewer Overflow
STORET	EPA Storage and Retrieval System
TMDL	Total Maximum Daily Load
TOD	RTI International Task Order Director
TOM	EPA Region 5 Task Order Manager
USGS	U.S. Geological Survey

DISTRIBUTION

This document will be distributed to the following team members involved in this project from U.S. Environmental Protection Agency (EPA) Region 5, MDEQ, RTI International, and ECT Incorporated. Additionally, sampling team members and anyone involved in the aspects of this project discussed in this document will receive a copy of the document.

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1.0 Project Objectives, Organization, and Responsibilities

The primary objective of this project is to collect *E. coli* and bacterial source data to be used in developing the Total Maximum Daily Load (TMDL) for *E. coli* in the Detroit and Ecorse River watersheds. This document is the quality assurance project plan (QAPP) for sample collection and conducting data assessment to support TMDL development.

Project Objectives

This QAPP provides a description of the work to be performed to support the development of a TMDL in the Detroit River and Ecorse River. This document also outlines procedures for collecting samples, sample handling, laboratory procedures, evaluating results, and data handling thus ensuring that the data used for TMDL development are scientifically valid and defensible and that uncertainty has been reduced to a practical minimum. An extensive amount of data and information will be collected to diagnose sources of *E. coli*, help quantify the level of existing impairment, and provide a foundation on which restoration activities may be identified and implemented. This QAPP will set forth the objectives, responsibilities, protocols, procedures, and methods for obtaining primary data.

Project Organization

The sampling organization is **Environmental Consulting & Technology, Inc**. (ECT). As such, ECT will be responsible for sample collection, handling, and delivery to the appropriate laboratories. ECT will also be involved with the project planning, and project management.

Paragon Laboratory will serve as the laboratory for *Escherichia coli* (*E. coli)* enumeration. Paragon staff will prepare the water samples and perform *E. coli* analysis using the EPA method 1103.1.

Source Molecular Corporation (SMC) has been selected to perform the bacterial source tracking analyses. The scientists at SMC are authorities on microbial source tracking, emphasizing genetic and molecular techniques.

Roles and Responsibilities

U.S. Environmental Protection Agency (EPA) Region 5 is funding this project, through a task order under EPA's Watersheds contract (no. 68-C-02-110) with RTI International; the Michigan Department of Environmental Quality (MDEQ) is providing technical advice and oversight. The RTI Team includes staff from Environmental Consulting & Technology, Inc. (ECT).

Julianne Socha will provide overall project/program oversight for this study as the EPA Region 5 Task Order Manager (TOM). Ms. Socha, along with Christine Alexander from the MDEQ will review and approve the QAPP, final report and draft TMDL work plan and ensure that all contractual issues are addressed as work is performed on this task order. Bill Cooter, the RTI task order director (TOD), will work with the EPA Region 5 TOM to ensure that project objectives are attained. Ms. Socha and Ms. Alexander will also be responsible for coordinating with contractors, reviewers, and others to ensure technical quality; and adhering to project objectives and contract requirements.

As Project Director, Mr. Cooter will supervise activities conducted under the contract. Dr. Sanjiv Sinha will perform ECT Project Director duties, including review of all products before submittal to RTI TOD. Ms. Annette DeMaria will carry out ECT Project Manager duties, including oversight of the site selection, QAPP submission, final report preparation, monthly reporting and field crew activities. Ms. Meghan McGahey will be responsible for QAPP development, data analyses, weekly data reporting and will lead the sampling effort. Mr. Marty Boote will be responsible for the TMDL work plan development.

2.0 Project Definition/Background

The Detroit River is located in southeast Michigan and acts as a natural boundary between part of the United States and Canada. The international boundary between the two counties runs down the River, leaving Belle Isle, Grassy Island, Zug Island and Grosse Ile on the U.S. side of the River. The river distance is approximately 32 miles from Lake St. Clair to Lake Erie (See Figure 1). The water- surface elevation falls approximately 3 ft within the River, which has an average discharge of about 186,000 ft^3/s (USGS p. 10).

The Detroit River receives discharges from numerous stormwater outfalls, industrial point sources, and several combined sewers (during heavy rains). The Rouge and Ecorse Rivers, as well as, Conner and Marsh Creeks discharge to the River from the U.S. side.

The Ecorse River drains 43 square miles of Wayne County, Michigan and is home to about 160,000 people. There are three primary watercourses within the Ecorse Creek watershed: the North Branch of Ecorse Creek extends 16 miles in the northern portion of the watershed, the LeBlanc Drain runs 9.6 miles and drains the central portion of the watershed, and the South Branch Ecorse Creek (also known as the Sexton-Kilfoil Drain) extends 16 miles draining the southern portion of the watershed. As of September 2004, the River was receiving discharges from 48 permitted point source discharges and numerous storm sewers (Ecorse Watershed Management Plan p. 2-1).

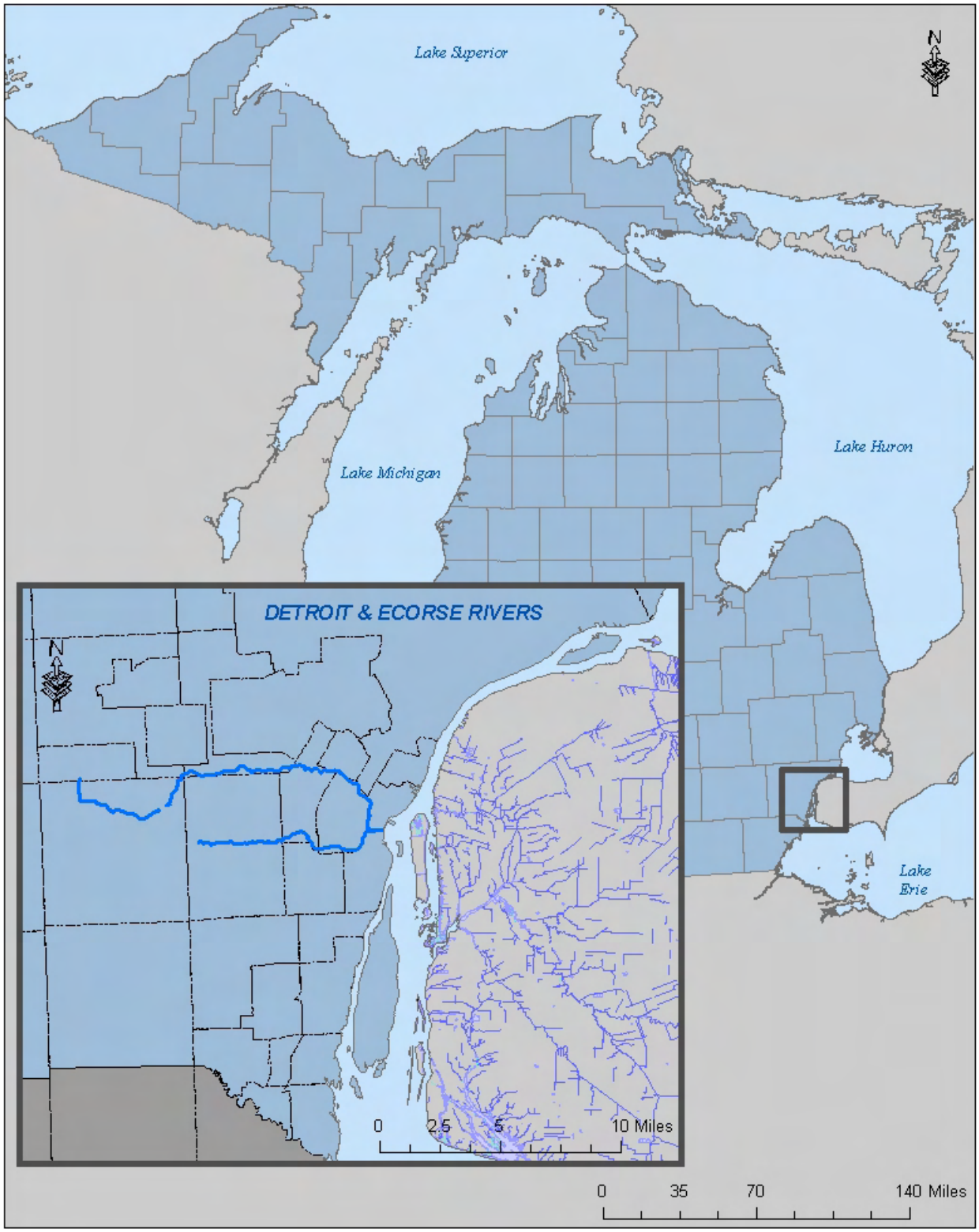
The water quality problems associated with the Detroit and Ecorse Rivers are summarized in Table 1.

Table 1. Detroit River and Ecorse River impaired segment as indicated on Michigan's approved 2006 303(d) List.

Reach	Description	River ID	Affected Uses	Pollutant/ Stressor
Detroit River	Entire River from Lake St. Clair to Lake Erie	061401 D	-Total body contact recreation	CSO, untreated sewage discharge, pathogens (Rule 100)
Ecorse River, North & South Branches	Beech-Daly Rd downstream to Dix-Toledo Rd & Inkster Rd. downstream to Electric Ave.	061301L	-Total body contact recreation	untreated sewage discharge, pathogens (Rule 100)

Part 4 of the Michigan Water Quality Standards, R323.1062 Microorganisms, requires that all waters of the state be protected for total body contact recreation and not contain more than 130 *E. coli* colonies per 100 mL, as a 30-day geometric mean. In addition, waters of the state that are protected for total body contact shall not contain more than 300 *E. coli* colonies per 100 mL, as a geometric mean of at least 3 samples collected during the same sampling event.

Figure 1. Project Area



3.0 Project Description

ECT will perform scheduled weekly sampling according to the procedures outlined in this document. Samples will be collected weekly for a period of 22 weeks. Samples analyzed for *E. coli* density. After the completion of several weeks of *E. coli* sampling, a subset of sites will be selected for DNA sample collection and analysis. The DNA samples will be analyzed to determine if the source of the *E. coli* is human/non-human in origin. A task by task description of this effort follows.

Task 1: Evaluate watersheds to select appropriate sampling locations

ECT worked with the MDEQ/EPA to select the specific monitoring sites. The *E. coli* sampling sites were selected based on several factors including personnel safety (i.e.: condition and width of shoulder, vehicular traffic conditions, etc), available access to the river (i.e.: there are at least nine public boat ramps along the Detroit River that can be used to launch a boat for sample collection activities on the River), the size and condition of the stream, sufficient depth and flow, the relative location to tributaries, the ability to return to the location by landmarks, and the availability of data necessary for TMDL development. These selected locations are further described in Appendix A.

Task 2: Conduct *E. coli* sampling

The selection of sampling procedures was based on field staff safety (i.e.: avoidance of confined space entries when storm sewer sampling is required), the holding time associated with the analysis, the ability to collect a representative sample, and the parameters being analyzed (i.e.: bacteria samples must be collected in sterilized containers).

ECT will perform scheduled weekly sampling according to the procedures outlined in this QAPP. Sampling will be conducted at the locations outlined in Appendix A. Samples will be collected from May 1–October 31, 2007 for a period of 22 consecutive weeks. Each sample location will be recorded using a global positioning system (GPS) to within one-meter horizontal accuracy.

There will be three separate sampling approaches employed for this project. When referring to "left" or "right" bank/side of the creek, orientation is always when looking upstream.

- Samples along Ecorse Creek will be collected from the upstream side of the bridge crossing where practical. Three samples will be collected at each of the seven selected crossing at the left, center and right portions of the stream, always starting at the left when facing upstream.
- A single grab sample will be collected from the LeBlanc Drain from street level using a sampling pole to lower the sterile laboratory bottle into the manhole. Samples will be collected from the center of flow. If no flow is present, no sample will be collected. In addition, grab samples will be collected from the LeBlanc Drain outlet at Council Point Park and from the Ecorse River at the West Jefferson Ave. bridge during wet conditions as long as flow is present.
- Samples from the Detroit River will be collected from the bow of a boat, when the boat is facing upstream. Samples taken from the boat will be collected by retrieving a minimum of three separate samples along the cross section of that river/creek/canal. The first sample collected will be within 200 feet of the left bank (US shoreline in most cases). The samples will traverse the cross section, with a minimum of three samples collected for each designated river/creek/canal cross section. The final sample for a cross section will be collected within 200 feet of the right bank (Canadian shoreline) where practical.

All samples will be collected directly into a sterile laboratory bottle suspended on a sampling pole. Samples will be collected in a manner that minimizes disturbance of the stream bottom so as to avoid capturing sediments. Latex gloves worn during sample collection will be changed between sampling sites. Field notes will be taken at each sampling site and will be recorded on the Sample Collection Data sheets. Notes will include date, time, any notable stream conditions, etc. In addition to the regular stream samples, duplicate and blank samples will be collected and sent to the laboratory for analysis. Duplicate samples will be collected every 10 samples and field blank samples will be collected every 20 samples.

Once sampling is completed, the samples will be stored in coolers at 4ºC and transported to Paragon Lab for analysis using EPA Method 1103.1. Samples will be transported to Paragon laboratory for analysis within five hours of collection.

Task 3: Make *E. coli* data available to all project partners, public, etc.

Prior to the onset of sampling, ECT will receive a login ID and password for the MDEQ's Beach Monitoring website [http://www.deq.state.mi.us/beach/public/default.aspx]. On a weekly basis, ECT will update the website with the previous week's data. ECT will bulk enter the data from an Excel spreadsheet provided by the laboratory. Before submitting the information, the person entering the data will check 10% of the entries for accuracy. The data will then be copied, pasted, and uploaded, thereby posting it to the website.

Additionally, ECT will submit the *E. coli* results electronically to the EPA/MDEQ in Excel® spreadsheet format, on a weekly basis. An example of this format is included in Appendix B. The data will be cumulatively entered into the spreadsheet and organized by watershed and by monitoring station. The table will be set up to automatically calculate the daily geometric means (and 30-day, if needed) and to highlight values above water quality standards. ECT will include a description of any problems encountered during sample collection, transport and analysis, any deviations from the QAPP, and any associated remedial actions taken to address the problems or deviations. The final *E. coli* (and DNA) data will be presented in graphical and table format for ease of interpretation.

ECT will submit the DNA data to the EPA/MDEQ within 4 days of receipt of the results. The data will be transmitted in electronic spreadsheet format and organized by monitoring station. ECT will include a description of any problems encountered during sample collection, transport and analysis, any deviations from the QAPP, and any associated remedial actions taken to address the problems or deviations.

Task 4: Conduct Quality Assurance checks on data

ECT will receive the *E. coli* analytical results from the laboratory in electronic MS Excel® format so as to eliminate any errors that may occur in data transcription from the lab report to the spreadsheet. Electronic reporting will also facilitate reporting to the MDEQ and the Beach Monitoring website. ECT will pre-screen the *E. coli* results for quality purposes. ECT will ensure that the laboratory reported results for each sample submitted and met the holding time and detection limit. Once the data passes the initial quality control (verification) check, it will be validated based on the procedures outlined in section 4 of this QAPP.

Task 5: Select sampling locations for DNA analyses

DNA sample locations will be a subset of the *E. coli* sample locations. The selection of the DNA locations should be based on *E. coli* concentrations (i.e.: it is prudent to select sites that have *E. coli* densities that are often above water quality standards) and the location of potential sources areas (i.e.: placing sites upstream and downstream of potential sources – WWTP, large storm sewers, combined sewers, etc. may provide some insight to what is occurring in the stream).

After completing about 6 weeks of sampling, ECT will consult with MDEQ and EPA to determine the locations for collection of samples for DNA analysis. Up to ten sites will be selected. Duplicate samples will then be collected from all three locations of each of the ten selected sites. Only one sample will be submitted for any given site. If weather permits, at least five samples will be collected during a wet weather event. If possible, sampling for DNA analysis will be submitted well before the end of the sampling period to ensure that DNA fingerprinting results will be received with sufficient time remaining to incorporate these results in the final report. The locations selected for DNA analysis are listed in Appendix E.

Task 6. Collect water samples for DNA analyses

The samples for DNA analysis will be collected as a split sample during the regular weekly sampling, so as to directly correlate the *E. coli* density to the *E. coli* source. The DNA water sample will be immediately shipped overnight (so as to reduce hold time and potential bacteria die-off) to the DNA laboratory, but not analyzed until the *E. coli* density results are determined. This will eliminate costly DNA analysis for samples that have geometric means below water quality standards.

DNA samples will be collected concurrently with the *E. coli* samples. When DNA samples are collected, the field team will collect a minimum of 200 ml of stream water in a sterile bottle. One hundred milliliters will immediately be transferred to a sterile 100 ml bottle for DNA analysis. The original bottle will be sent for *E. coli* enumeration as described previously. The DNA samples will be placed with ice in a cooler and shipped by overnight courier to the Source Molecular facility in Miami, Florida. Source Molecular will analyze the samples using both the human Enterococcus and human Bacteriodetes identification methods. The result of the analysis for each sample will be a determination of whether the source of the *E. coli* is human or nonhuman in origin.

Task 7: Develop draft work plan for TMDL development

Although the data collected for this project is intended for TMDL development, the actual TMDL document is not covered under the scope of work for this project. Therefore, details regarding the TMDL formation are not covered in this document.

However, ECT will develop a draft work plan that identifies available approaches for developing *E. coli* TMDLs. The purpose of the draft TMDL work plan is to outline how an *E. coli* TMDL can be developed for the Detroit and Ecorse Rivers, and is based upon a thorough review and assessment of available water quality data, existing watershed conditions, and available methods and predictive tools.

ECT will review available literature, other completed bacteria TMDLs, and guidance documents to identify methods, predictive tools, and models for estimating loadings, setting numeric TMDL targets, establishing linkages between pollutant sources and in-stream *E. coli* concentrations, and determining an appropriate margin of safety. This review will result in a list of potential approaches to developing the *E. coli* TMDL and an assessment of the different approaches. ECT will provide a recommended approach based on its assessment of water quality data, potential sources, and available approaches.

ECT will conduct an *E. coli* source assessment using GIS tools and available spatial data in addition to existing management plans, reports, and databases. The assessment will identify the distribution of various land uses and land types within the watersheds on a subwatershed basis. Subwatersheds will be based on existing GIS data available through the Michigan Spatial Data Library and other watershed modeling efforts. The watershed assessment will include research of existing NPDES permits, watershed management plans, outfall databases, identified illicit discharges, and other potential sources of information that may lead to identification of *E. coli* sources. Spatial and temporal patterns in water quality will also be considered. The *E. coli* source assessment will result in a list of potential and likely sources of *E. coli* throughout the watersheds.

ECT will research and identify potential TMDL implementation strategies. The purpose of this research is to outline and assess potential implementation strategies and existing frameworks. This aspect of the work plan will require identification of stakeholders; assessment of existing governmental frameworks; summarization of on-going storm water management, CSO, SSO, and illicit discharge activities; and evaluation of existing watershed management plans.

The general schedule for deliverables under this project SOW is presented in Table 2. Project activities include producing and then finalizing the QAPP, writing draft and final reports on the sampling results, and developing the TMDL draft work plan.

Table 2. Schedule for Deliverables.

Action/Deliverable	Due date
Project Kickoff Conference Call	January 24, 2007
Sample Site Selection	February 12, 2007
QAPP delivered to EPA and MDEQ (Draft)	February 23, 2007
Participate in stakeholder meeting and request feedback on the sampling locations	March 22, 2007
QAPP delivered to EPA and MDEQ (Final)	April 6, 2007 (within 7 days of receipt of EPA comment)
QAPP approved by EPA	Mid to late April 2007
Revised QAPP delivered to EPA and MDEQ to include DNA sampling locations	June 22nd
Monthly progress reports	January 2007 to January 2008 (by the 5th of the month)
Weekly *E. coli* sampling	May 1 to October 31, 2007
DNA sampling and analysis	July 1 to September 14, 2007
Weekly sampling results input into website and reported to MDEQ	May 11 to November 2, 2007
TMDL workplan (Draft)	October 19, 2007
Sampling Final Report (Draft)	November 21, 2007
Sampling Final Report (Final)	December 31, 2007

4.0 Quality Objectives and Criteria

The primary objective of this project is to collect data of a known quality that comply with MDEQ rules for surface water quality monitoring programs to support the development of a Total Maximum Daily Load (TMDL) for microbial contaminants in the Detroit and Ecorse River watersheds. To achieve this objective, *E. coli*, the bacterial indicator organism identified in the Michigan water quality standards, will be measured at several locations in the project area. In addition, bacterial source tracking samples will be collected to identify sources of bacterial contamination (human or non human) within the targeted drainage area.

A mixture of laboratory and field variables may affect data quality. The variables include sample matrix variability, sample collection/handling procedures and equipment, sample analysis techniques and record keeping. To control these variables, the Data Quality Objective (DQO) process is used. DQOs developed for this project specify discrete parameters in six areas: Precision, Accuracy, Representativeness, Comparability, Completeness and Sensitivity (PARCCS). A brief description of each of these parameters is presented below, along with the formulas for calculation of precision, accuracy and completeness for the scheduled analyses.

Precision and completeness are expressed and evaluated quantitatively. Representativeness, accuracy, comparability and sensitivity are more subjective in nature and are addressed in both quantitative and qualitative terms. The primary QA objective is to measure the quantity of target analytes in each sample without unacceptable bias.

Note: There are no specific quality control measurement required of Source Molecular Laboratories by EPA or MDEQ aside from routine laboratory quality control measurements specified in Parveen, et.al., 1999. For details, refer to the complete ribotyping method included in Appendix D.

Ambient Water Reporting Limits

The laboratory will not perform ambient water reporting limit (AWRL) verification for the *E. coli* tests. The AWRL for *E. coli* results is normally 1 colony/100mL; however, the actual reporting limit may vary from sample to sample depending on the density of the organisms in a sample and the number of dilutions employed for each sample.

Precision

Precision is determined as a measurement of the closeness of individual test results under prescribed conditions, and reflects a combination of random and systematic error, as well as natural variation within a specific matrix. Only data generated within the required precision criteria will be deemed usable. However, the Laboratory QC Manager, prior to rejecting data as unusable, will closely evaluate the data for potential matrix interference and its effects on the results.

The precision of measured data is affected by natural variability in the sampling matrix as well as laboratory and sampling factors. Laboratory precision will be assessed through the analysis of laboratory control samples, as well as by initial and continuing calibration of instrumentation. In addition, a semi-quantitative evaluation of laboratory precision will be assessed through analysis of field duplicates submitted as blind samples to the laboratory.

Field precision or the ability of the sampling team to collect two samples with a high degree of similarity, may also be assessed by the collection and submission for analysis of field duplicate QC samples. Field duplicate samples are collected from the same location, at the same time, using the same sampling method, and independently analyzed in the same manner.

For quantitative microbiological analyses, the method used for calculating precision is the one outlined in Standard Methods for the Examination of Wastewater, 18th Edition, section 9020 B.4.b:

$$RPD_{bacteria} = (\log X_1 - \log X_2)$$

The $RPD_{bacteria}$ should be lower than $3.27 \sum R_{log} / n$, where R_{log} is the difference in the natural log of replicate samples for the first or most recent set of 15 samples, and where n is the number of samples.

Accuracy

Accuracy measures the bias in a measurement system. Accuracy cannot be directly measured for bacterial samples. Accuracy will be assessed by the laboratory through the analysis of positive and negative controls.

Representativeness

Representativeness is an expression of the extent to which measured data accurately represents actual conditions. The objective of this sampling effort is to collect samples that accurately represent conditions in the field. The careful design of the sampling plan is of paramount importance in ensuring that the data are representative of prevailing conditions. The sampling plan specifies the number and location of samples to be collected.

The key factors considered in the design of the sampling plan included: (1), providing a sufficient number of samples, and (2) sufficient spatial distribution of samples to ensure that the target area is covered.

Finally, representativeness is dependent on using appropriate sample collection, handling, and analysis procedures. These procedures are described elsewhere in this document.

Completeness

Every effort will be made to obtain valid data for each sampling point at all times. Completeness will be measured by dividing the number of planned usable sample results to the total number of sample results. The completeness objective for this project is for 90% of the planned data to be usable (samples collected and analyses generated within the established control limits for precision and accuracy). Completeness is calculated as:

$$\%C = (V/T) * 100\%$$

Where

V = Number of measurements judged valid

T = Total number of samples analyzed

Comparability

In order to maximize the degree of comparability of data generated for this project with previous sampling and analysis program results, sampling locations will, whenever possible, correspond to locations used in the foregoing studies. Sample collection methods, holding times, sample preservation and laboratory analysis methods will all be conducted in accord with specified standard methods and protocol. The object is to facilitate observations and conclusions that can be directly compared with historical and/or available background data.

Sensitivity

Sensitivity is a term broadly applied to the minimum detection capabilities of the specified methods of analysis and instruments used to conduct the scheduled analyses. Minimum detection limits and practical quantitation limits must be established to assure that the selected method of analysis is sensitive enough to detect and quantify concentrations for the parameters of interest. The method description provides a discussion of the Method Detection Limits (MDLs) for the procedure. These limits have been reviewed and judged to be adequate for the purposes of this study.
Another variable that may affect sensitivity is holding time. Each analytical procedure has a designated maximum holding time from the point of sample collection to extraction and analysis in the laboratory. The maximum holding time for each analytical parameter is listed in the corresponding method's specific SOP.

Adequate sensitivity in the project data will be verified through a comparison of the reported PQLs after analysis to those in the method's SOPs. Holding times will likewise be compared to the maximum time specified in each method specific SOP.

5.0 Special Training Requirements

No special training requirements or certifications are necessary for the completion of this work. It is advisable that individuals selected to be sampling team members have received appropriate health and safety training that is typical for environmental professionals.

6.0 Documentation and Records

Sampling information will be recorded on sample collection data sheets. The sheets have been prepared specifically for water quality sampling. A copy of the sample collection sheet is included in the Sample Collection and Handling SOP; in Appendix B. Sampling sheets are completed on-site at the time sampling occurs. Project staff shall retain sampling collection records and all

records of field activity for five years following completion of the project. Additionally, all field records will be submitted to the MDEQ with the final report.

The laboratory will provide data in electronic format. The handling of data from this point on, is discussed in sections 16.0 – Data Management, 18.0 – Reports to Management, and 19.0 – Data Review, Verification, & Validation Methods.

The RTI Team will prepare monthly progress reports that will address task and subtask milestones, deliverables, adherence to schedule, and financial progression at the end of each full month while the task order for this project is open. Data and assumptions used to develop the TMDL models will be recorded and provided to EPA for inclusion in the TMDL report.

The RTI Team will maintain a project file, which will act as a repository for all field logs, sampling data and any additional information used to develop the TMDL workplan. This file will be maintained for at least five years (unless otherwise directed by the EPA Region 5 TOM). Electronic project files will be maintained on network computers and backed up periodically. The ECT Project Manager will supervise the use of materials in the project file. If requested by EPA, RTI will provide this information in an administrative record at a later date.

The following information will be included in the hard copy or electronic project files in the central file:

- All EPA-approved versions of the QAPP
- Any reports and documents prepared
- Contract and task order information
- Electronic copies of laboratory reports
- Results of data quality assessments and audits
- Communications (memoranda; internal notes; telephone conversation records; letters; meeting minutes; and all written correspondence among the project team personnel, subcontractors, suppliers, or others)
- Maps, photographs, and drawings
- Studies, reports, documents, and newspaper articles pertaining to the project
- Spreadsheet data files: physical measurements, analytical chemistry data (hard copy and on CD).

Copies of formal reports generated from the data and submitted to EPA will be maintained in the central file (diskette and hard copy) at RTI's Research Triangle Park, NC office. The data reports will include a summary of the types of data collected, sampling dates, and any problems or anomalies observed during sample collection.

7.0 Sampling Process Design

Sampling will be conducted at 9 cross sections on the Detroit River (for a total of 57 samples), two locations on the LeBlanc Drain, and 7 cross sections on the North and South Branches of Ecorse Creek (for a total of 21 samples). In addition, a grab sample will be taken from the outlet of the LeBlanc Drain during wet conditions if flow is present. The EPA and MDEQ have approved all sampling locations. More detail on these locations can be found in Appendix A. Sampling will be completed using manual sampling techniques as described in Section 3.0. Samples will be collected weekly at each location for 22 consecutive weeks from May 1, 2007 – October 31, 2007.

8.0 Sampling Methods

ECT will collect a minimum of 3 samples at each site during the weekly sampling event. A summary of the number of samples that will be collected is shown in Table 3. The sampling

procedure is described below. Additional details on the sampling procedures can be found in the Standard Operating Procedures found in Appendix B.

Table 3. Frequency and Type of Samples Collected

Parameter	Lab	Total number of samples per event*	Total Number of Samples
E. coli samples	Paragon	81	1782
QA samples	Paragon	8 duplicates 4 blanks	264
Human Enterococcus ID	SMC	To be determined	10
Human Bacteroidetes ID	SMC	To be determined	10

*Does not include any wet samples from sites EC0 or EC9.

ECT will submit samples to Paragon laboratory within 5 hours of sample collection in order to maintain sample hold time. ECT will make multiple trips to the laboratory to drop off the samples.

More details on how communication with the lab will be coordinated before, during, and after sampling events can be found in the Laboratory Coordination SOP, in Appendix B.

Sampling

A minimum of 100mL of sample water will be collected for each sample to be submitted from each site, leaving a minimum of approximately 1 inch of headspace in the sample bottle. Samples will be collected using a sampling pole, and the water will be collected directly into the laboratory bottle. For duplicate samples, a minimum of 200mL of sample water will be collected. The sample will be collected in one 300 mL sterilized container. The sample will be well mixed, and then the sample will be split into two sterile laboratory bottles. One bottle will be labeled as normal, the other will be designated as a duplicate sample as described later in this section. In addition to duplicate samples, blank samples will also be submitted for analysis. A blank sample will be collected by pouring distilled water directly into the sterile laboratory bottle. This sample will be labeled as described later in this section. All samples will be stored in a cooler with ice, maintained to approximately 4°C. This cooler will be taken to the Paragon Laboratory for *E. coli* analysis.

When collecting samples for DNA analysis, this sampling procedure will change slightly. Samples will still be collected for *E. coli* analysis, however, up to 10 sites will also have samples to be potentially submitted for DNA analysis. ECT/RTI, MDEQ, and the EPA will select these sites after several weeks of *E. coli* sampling. The sites identified for DNA sample collection are listed in Appendix E. A minimum of 300mL of sample water will be collected at each location at each site that is selected for DNA analysis. Samples will be collected in one 300 mL sterilized container. The sample will be well mixed, and then the sample will be split into the required number of laboratory bottles (at least 2, 3 for duplicate samples). One bottle from each location at each site will be stored in a cooler with ice. The second bottle from each location will be stored in a separate cooler on ice. One cooler will be taken to Paragon Laboratory for *E. coli* analysis. ECT will ship all of the samples in the second cooler via an overnight courier service to Source Molecular Corporation for DNA typing analysis. Upon receiving the *E. coli* data from Paragon Laboratory, ECT will notify Source Molecular Corporation which samples (1 per site) to analyze for the Human Enterococcus ID and Human Bacteroidetes ID.

More details can be found in the SOPs, found in Appendix B.

Decontamination
The samples will be collected directly into sterile laboratory bottles; therefore, decontamination will not be necessary.

Sample Labeling
All sample bottles will be pre-labeled on the bottle rather than on the cap to identify the sample for laboratory analysis. Sample labels will include type of sample, sampler's name, date, time, and location.

Sample identification will use the following Sample Numbering Scheme:

NN#SEV - d/b

Where:
NN# = Station Name/Location Identifier
DR = Detroit River
EC = Ecorse River/LeBlanc Drain
# = Site number
S = Sample (A, B, C, …) Always beginning with "A" on the left bank when facing upstream.
EV = Number of Event (01, 02, 03…22)
d = Field duplicate sample (this is to be left blank if the sample is not a duplicate sample)
b = Field blank sample (this is to be left blank if the sample is not a blank sample)

Example #1: DR8C06
This sample is from the Detroit River, collected from cross section #8. It is collected during the sixth week of sampling, and is the third sample collected from the left side of the river, when facing upstream.

Example #2: DR8C06 - d
This sample is from the Detroit River, collected from cross section #8. It is a duplicate of the sample collected during the sixth week of sampling, the third sample collected from the left side of the river, when looking upstream (duplicate of Example #1).

Example #3: EC5A02 – b
This sample is a blank sample, designated as being collected during week 2 on Ecorse River at site 5.

The chain of custody form has a column for indicating whether a sample was a grab sample or a composite sample. All samples are to be indicated as grab samples.

Equipment Blanks
Equipment blanks will be collected every 20 samples. Field crews will collect one additional sample, using deionized (DI) water. The equipment blanks will be submitted to the laboratory for analysis with the event samples.

Duplicate Samples
Duplicate samples will be collected 1 in every 10 samples. Field crews will collect one additional sample, by splitting one oversized sample into 2, so as to collect from the same pool of water. The duplicate samples will be submitted to the laboratory for analysis with the event samples.

9.0 Sample Handling and Custody

Chain-of-custody procedures will be followed to provide documentation of the handling of each sample from time of collection through receipt by the laboratory. Each laboratory shall provide chain-of-custody forms to be filled out by the sampler/sample team leader to accompany each

sample through transit from the field to the laboratory. This form is used by both the field sampler and the laboratory to verify the contents of each shipment of samples. When transferring possession of the samples, both the individual relinquishing the container(s) and the receiver are required to sign and date the chain-of-custody form.

Upon receipt of the shipment at the laboratory, the contents of the cooler are checked against the completed chain-of-custody form. Any anomalies are to be immediately reported by the laboratory to the sampling team leader for clarification/resolution.

10.0 Analytical Methods

Water samples will be analyzed using established methods as summarized in Table 4.

Table 4. Number and Type of Samples Analyzed by Parameter

Parameter	Lab	Method Number	Method Detection Limit (CFU/100mL)	Sample Volume (mL)	Bottle Type	Hold Time	Number of Samples
E. coli enumeration	Paragon	EPA 1103.1	Lower: 1 Upper: 2,000,000	100	P	6 hours	*2046
Human Enterococcus ID	SMC	EPA 1600	NA	100	P	NA	10
Bird Enterococcus ID	SMC	EPA 1600	NA	100	P	NA	10

NA = Not applicable
P = Plastic
* Includes QA/QC samples, but does not include any wet samples from sites EC0 or EC9.

11.0 Quality Control

Sampling Quality Control Requirements and Acceptability Criteria

The minimum Field QC requirements are outlined in the Michigan's Surface Water Quality Division's quality assurance manual titled, "Quality Assurance Manual for Water, Sediment, and Biological Monitoring", 1994. The field staff should complete a documented review of 100% of the field data for compliance with QC requirements and ECT Quality Assurance Officer will complete a documented review of a minimum of 10% of the field data. Specific requirements are outlined below. Field QC Samples are reported with the weekly data report.

Bottle Blanks - A field blank is a sample of reagent water poured into a sample bottle. It is collected in the same type of container as the environmental sample, preserved in the same manner and analyzed for the same parameter. In addition to regularly collected bottle blanks, laboratory equipment blanks are prepared at the laboratory where collection materials are cleaned between uses. These blanks document that the materials provided by the laboratory are free of contamination. The QC check is performed before each set of equipment is sent to the field, and before each new batch of samples to be analyzed in the laboratory. The analysis of field blanks should yield values less than the AWRL. When target analyte concentrations are very high, blank values must be less then 5% of the lowest value of the batch.

Field duplicates - A field duplicate is defined as a second sample (or measurement) from the same location, collected in immediate succession, using identical techniques. This applies to all cases of routine surface water collection procedures, including in-stream grab samples and other water sampling devices. Duplicate samples are sealed, handled, stored, shipped, and analyzed in the same manner as the primary sample. Precision of duplicate results is calculated by the relative percent deviation (RPD) as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X1 and X2, the RPD is calculated from the following equation:

$$RPD = \{ (X1 - X2) / [(X1+X2)/2] \} * 100$$

For bacteria, the logarithms of the actual counts are used in this formula. Field duplicates will be collected at a frequency of 10% or greater.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

Detailed laboratory QC requirements are contained within each individual method and Laboratory Quality Assurance Manuals. The minimum requirements that all participants abide by are stated below. Lab QC sample results are reported with the data report.

Laboratory duplicate - Laboratory duplicates are used to assess precision. A laboratory duplicate is prepared by splitting aliquots of a single sample (or a matrix spike or a laboratory control standard) in the laboratory. Both samples are carried through the entire preparation and analytical process. Laboratory duplicates are analyzed on 10% of samples analyzed. Acceptability criteria are outlined in Section 4.0.

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the laboratory. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair. Precision limits for bacteriological analyses are defined in Section 4.0. Performance limits and control charts are used to determine the acceptability of duplicate analyses.

Method Blank- A method blank is an analyte-free matrix to which all reagents are added in the same volumes or proportions as used in the sample processing and analyzed with each batch. The method blank is carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the Minimum Analytical Level. For very high level analyses, blank value should be less then 5% of the lowest value of the batch.

Control Cultures – Each new lot of modified mTEC media is tested with positive control cultures (known *E. coli*) to verify that it supports growth of *E. coli*, and negative control cultures (known non-*E. coli* bacteria) to ensure the selectivity of the media.

Colony Verification - For initial test of analyst capability, and with each new batch of media, ten red or magenta presumed *E. coli* colonies from one or more completed tests are verified as *E. coli* through procedures described in the method.

Failures in Quality Control and Corrective Action

Sampling QC excursions are evaluated by the ECT Project Manager, in consultation with the MDEQ and EPA. In that differences in field duplicate sample results are used to assess the entire sampling process, including environmental variability, the arbitrary rejection of results based on predetermined limits is not practical. Therefore, the professional judgment of the ECT Project Manager and ECT Quality Control Officer will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility. Notations of field duplicate excursions and blank contamination are noted in the weekly report and the final QC Report.

Corrective action will involve identification of the cause of the failure where possible. Response actions will typically include re-analysis of questionable samples. In some cases, a site may have to be re-sampled to achieve project goals.

12.0 Instrument/Equipment Testing, Inspection, and Maintenance

All instruments, and equipment will be inspected and tested for appropriate use.

13.0 Instrument/Equipment Calibration and Frequency

The laboratories will perform calibration of the instruments, as needed, by following any manufacturer's instructions.

14.0 Inspection/Acceptance of Supplies and Consumables

All sample bottles will be inspected prior to use. Tamper proof seals shall be fully in tact, or the bottle will be discarded.

15.0 Non-Direct Measurements

Non-direct data can be published or unpublished and can come from a number of sources, but the non-direct data most often used in TMDL modeling projects are typically obtained from the USGS stream gauge database, EPA's Storage and Retrieval System (STORET), EPA's Permit Compliance System, and databases maintained by state agencies. Stream flow data collected by the USGS may be used to assist in estimating loads of the target parameter. These data will be obtained from the USGS web site. These data are considered provisional for some time after their collection, generally until the publication of the annual water summary. Because the intended use of the data is only to explore the potential magnitude of pollutant loads in runoff, these data will be satisfactory. If these data were to be used to set permit limits or load allocations, the flow measurements will only be used once the provisional qualifier has been removed.

16.0 Data Management

On a weekly basis, ECT will submit the *E. coli* results electronically to the EPA/MDEQ in Excel® spreadsheet format, an example of this format is included in Appendix B. The data will be cumulatively entered into the spreadsheet and organized by watershed and by monitoring station. The table will be set up to automatically calculate the daily and monthly geometric means and to highlight values above water quality standards. ECT will include a description of any problems encountered during sample collection, transport and analysis, any deviations from the QAPP, and any associated remedial actions taken to address the problems or deviations. The final *E. coli* (and DNA) data will be presented in graphical and table format for ease of interpretation.

Prior to the onset of sampling, ECT will receive a login ID and password for the MDEQ's Beach Monitoring website [http://www.deq.state.mi.us/beach/public/default.aspx]. On a weekly basis, ECT will update the website with the previous week's data. ECT will bulk enter the data from an Excel spreadsheet provided by the laboratory. Before submitting the information, the person entering the data will check 10% of the entries for accuracy. The data will then be copied, pasted, and uploaded, thereby posting it to the website.

ECT will submit the DNA data to the EPA/MDEQ within 4 days of receipt of the results. The data will be transmitted in electronic spreadsheet format and organized by monitoring station. ECT will include a description of any problems encountered during sample collection, transport and analysis, any deviations from the QAPP, and any associated remedial actions taken to address the problems or deviations.

17.0 Assessment and Response Actions

The following table presents the types of assessments and response action for data collection activities applicable to the QAPP.

Table 5. Assessments and Response Actions

Activity	Responsible Party	Scope	Response Requirements
Project Status, updates, and oversight	RTI and ECT	Monitoring of the project status and records to ensure requirements are being fulfilled. Monitoring and review of contract laboratory performance and data quality.	Report to EPA and MDEQ on a monthly basis via reports. Ensure project requirements are being fulfilled.
QAPP Submission to EPA and MDEQ	ECT	Develop plan for sample collection, data handling, and reporting for TMDL development.	Provide draft and final documents on schedule. Coordinate with EPA and MDEQ on technical questions and document edits.
Data Assessment Summary	ECT	Prepare and submit an assessment of all sampling data.	Provide draft and final reports on schedule. Coordinate with EPA and MPCA on technical questions and document edits.
Source Identification Summary	ECT	Prepare and submit a summary of potential sources contributing to the impairment.	Provide draft and final reports on schedule. Coordinate with EPA and MPCA on technical questions and document edits.
TMDL workplan	ECT	Prepare and submit a TMDL workplan that includes all applicable elements needed for TMDL development.	Provide draft workplan on schedule. Coordinate with EPA and MPCA on technical questions and document edits.

If problems arise in the process of completing the aforementioned activities, the RTI project manager will determine the appropriate long-term or short-term action to be taken. Steps to address the problem could include: investigation and determining the cause of the problem, implementing a corrective action, following-up with team members to ensure that the appropriate corrective action has been taken and that the problem has been resolved. If these steps do not adequately address the problem, the Project QC Officer will be responsible for corrective action and will inform the RTI Team TOD as appropriate.

18.0 Reports to Management

Monthly progress reports will be provided by RTI to the EPA Region 5 TOM. These progress reports will describe the status of the project and work completed as well as anticipated work to be completed during the next reporting period.

Two types of laboratory QA reports will be generated for this project, regular and problem specific. A regular QA report will be prepared by the Laboratory QC Manager for each batch of samples received and analyzed. This report will verify documentation of all in control results and summarize all out of control results. Out of control sample results will require corrective action as specified in the method specific SOP, and may include resampling and reanalysis.

The Laboratory QC Manager is responsible for maintaining surveillance of analysis procedures and results and for promptly identifying and correcting anomalies. All significant data quality problems/issues are to be promptly reported to the ECT Project Manager for resolution.

19.0 Data Review, Verification and Validation methods

All data to be used in the development of the TMDL will be reviewed for completeness and correctness. Raw data that is received in electronic format will be screened using visual inspection of the data (scanning for values outside of the typical ranges) and electronically (data statistics in Microsoft Excel®). Any data received in hard copy will be assessed by comparing the original data to the entered electronic data. Additional steps for data evaluation are discussed in Sections 4.0, 11.0 and 17.0.

All sampling results will be crosschecked against the field notebook, sample tags, and chain of custody documents to ensure that the data summary is correct. Analytical results will also be compared to the chain of custody documents to ensure that the data are complete. The Project QA/QC Coordinator will also review the data to determine if it meets the QAPP objectives. Preliminary decisions to accept or qualify data are made by the Project Leader and Project Manager.

20.0 Reconciliation with user Requirements

The data quality objectives described in section 2 of this document are deemed to be consistent with and support the intended use of data set forth in the same section. ECT representatives will evaluate data continuously during the life-term of the project to ensure that they are of sufficient quality and quantity to meet the project goals. These data may be subsequently analyzed and used by the MDEQ for TMDL development, stream standards modifications and water quality assessments. If the data do not meet the goals specified in section 2 of this document, they will not be transferred to the statewide database to ultimately be used in decision-making. The evaluation of this data for decision-making is not part of this QAPP.

Literature Cited

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APPENDIX A

Sample Site Locations

Sample Site Locations

Detroit River cross sections

Multiple samples will be taken at each cross section as indicated in each site description below. For each cross section, the left bank sample (looking upstream) and the right bank sample will be taken within 200' of the shoreline, unless otherwise noted.

Site ID	Description
DR0.	Detroit River at Windmill Pointe Park and upstream of Peche Island (5 samples; A - E).
DR1.	Detroit River just downstream of Conner Creek and upstream of Belle Isle (5 samples; A - E).
DR2.	Scott Middle Ground near Detroit Boat Club – (3 samples; A - C); and Fleming Channel – south side of Belle Isle, (3 samples; D - F).
DR3.	Detroit River downstream of Renaissance Center (5 samples; A - E).
DR4.	Detroit River at Fort Wayne (5 samples; A – E.)
DR5.	Detroit River downstream of Zug Island at Rouge Edison Power Plant (left bank sample within 50' of large steel circular mooring cribs) (5 samples; A - E).
DR6.	Detroit River downstream of Ecorse River (5 samples; A - E); and Detroit River between the northern end of Fighting Island and Canadian mainland, upstream of Canadian Grassy Island (3 samples; F - H).
DR7.	Trenton Channel near Elizabeth Park (3 samples; A - C); and Livingstone Channel upstream of Stoney Island between Grosse Ile and unnamed wedge-shaped island (6 samples; D - I).
DR8.	Detroit River west of Celeron Island, left bank sample to be taken north of Lake Erie Metro Park boat ramp (3 samples; A - C); and Detroit River southeast of Celeron Island (6 samples; D - I).

Ecorse Creek

EC0. Ecorse River @ West Jefferson Ave. (only during wet conditions, if flow is present)
Sample A.

NOTE: It was determined that the boat sample for DR6A is collected within 50 feet of the EC0 location, and therefore EC0 is a redundant location.

EC1. Ecorse Creek @ Fort Street, just south of Fort/Outer Drive intersection.
Samples A – C.

EC2. Ecorse Creek @ Pelham Rd, just north of Van Born Rd.
Samples A – C.

EC3. Ecorse Creek @ Beech Daly, south of Amherst St., west of Beech Daly. Same location as USGS gauging station #04168580.
Samples A – C.

EC4. Ecorse Creek @ Smith Rd; located between Middlebelt and Merriman on Smith. Just West of Kenwood St.
Samples A – C.

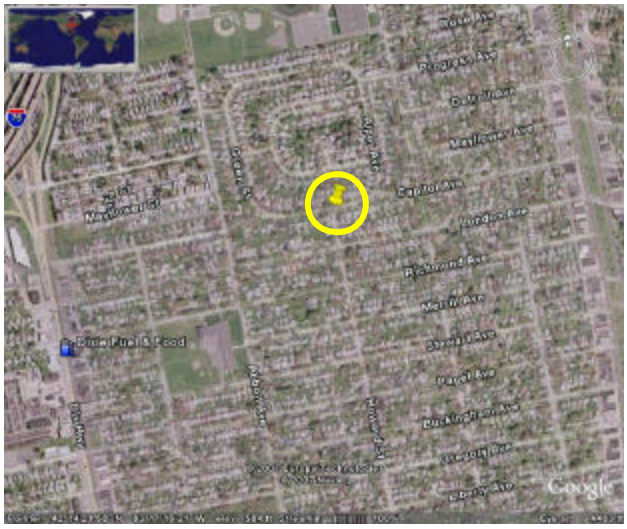
EC5. South Ecorse Creek @ Fort Street, just north of Fort/Goddard Rd intersection. Access from gas station at NW corner of intersection.
Samples A – C.

EC6. South Ecorse Creek @ Pelham Rd, located just north of Pelham/Goddard Rd intersection. Access from vacant gas station on west side of Pelham.
Samples A – C.

EC7. South Ecorse Creek @ Beech Daly, located north of Beech Daly/Goddard intersection. Access from east side of road.
Samples A – C.

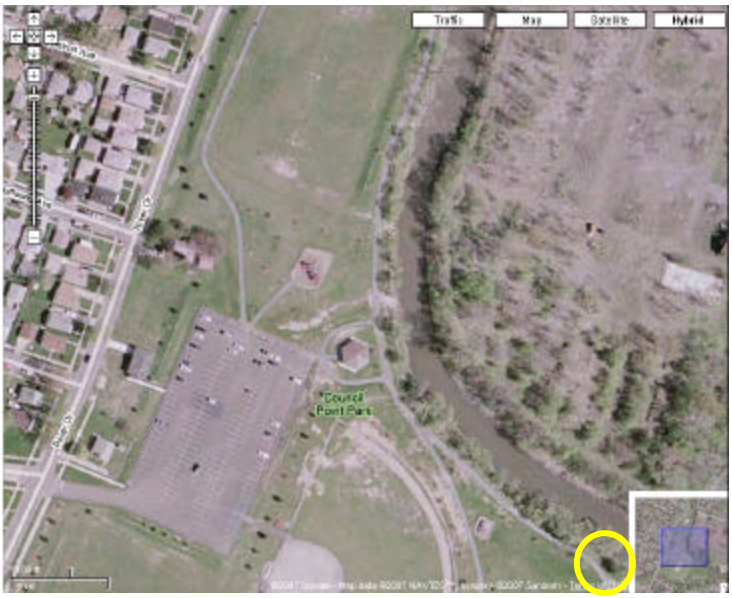
<u>LeBlanc Drain</u>

EC8. Capital Rd. & Howard, three blocks west of Fort St on Capitol. Capital is between Goddard and Champaign on Fort. Two manholes; one in the center of the road and the other on the south edge of the road.
Sample A-B.

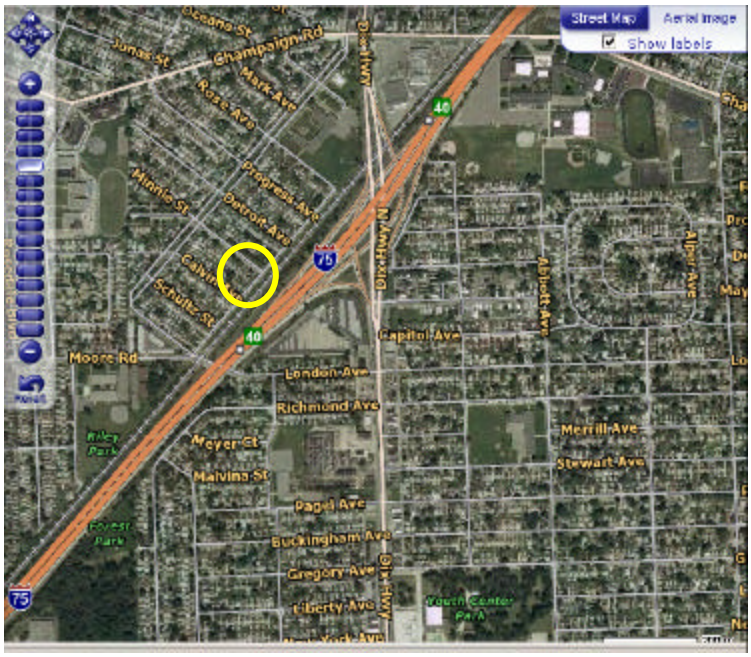


NOTE: Sampling was discontinued on EC8B after week 8, as it was discovered to have an illicit connection just upstream of the sampling point. EC10 was added at this point.

EC9. LeBlanc Drain outlet at Council Point Park, east of red, white and blue monuments (only during wet conditions, if flow is present). Sample A



EC10. Hartwick Rd. & N Wayne St. Hartwick is between Goddard and Champaign. Two manholes; Both are in the road. Sample A-B.



APPENDIX B

Environmental Consulting & Technology, Inc.
Standard Operating Procedures

Laboratory Coordination

Standard Operating Procedures

1.0 Scope and Application

This SOP covers the interaction between field staff and laboratories. The coordination issues between the field and the laboratories include: event notification; analysis requirements; expected number of samples; identification of event; laboratory bottle preparation, ordering, and management.

2.0 Method Summary

Two laboratories are contracted to analyze bacteriological samples collected during all sampling events: In-House Laboratory and Source Molecular Corporation. The laboratories have assigned a laboratory coordinator for the project.

The coordinator is responsible for reviewing internal QA/QC results generated by the lab to ensure the analyses are being conducted as specified in the QAPP. Procedures and forms have been established for ordering bottles.

3.0 Safety, Restrictions and Limitations

3.1 Safety
The laboratory bottles to be used for sample water collection will not contain any type of preservative. However, sample containers are sterile and all personnel shall always where protective latex gloves when handling sample containers.

3.2 Restrictions
None

3.3 Limitations
None

4.0 Sample Collection, Handling and Preservation

Covered in the Sample Collection and Handling SOP.

5.0 Equipment and Materials

A series of sterile sample bottles of a specified volume, material, and preservative.

6.0 Procedures

6.1 Laboratory Notification of upcoming events

Laboratory notification will be given at least 1 day in advance of an anticipated event. The task manager or alternate manager will notify the laboratory coordinator. The following information will be provided to the laboratory coordinator: Anticipated event start time, anticipated quantity of samples, anticipated time of sample availability. A form has been included with this SOP to be used for notification. It should be faxed or emailed to the lab coordinator at least 1 day before the sampling.

6.2 Laboratory bottle supply

a. The laboratory will supply all sample bottles. A bottle ordering form is attached to this SOP. Laboratory bottles must be ordered using this form.

b. Laboratory bottles will be kept in stock for at least 2 sampling events.

c. Reordering of laboratory bottles will occur every two weeks. Reordering shall be the responsibility of the field lead. Bottle orders should be routed directly (faxed) to the laboratory. Copies of bottle orders should be kept on file.

6.3 Laboratory bottle management

Bottles must remain capped and sealed until sample collection to maintain sterility. Laboratory bottles must be ready for sampling before the event starts, and will be inventoried for ordering following each event on the chain of custody (COC).

6.4 Other bottle supplies

Additional bottles may be required for grab sampling in the field, duplicate sampling containers, or other uses. Other bottle supplies will be ordered through the laboratory or another bottle supply source. The task manager should make bottle orders according to needs, and should allow a one-week delivery time. All bacteriological samples will be collected/handled in <u>sterile</u> bottles provided by the laboratory.

MEMORANDUM

To: **Sharon Johnson, Paragon Laboratory, Inc.; Thierry Sam Tamers, Source Molecular Corporation; Christine Alexander, MDEQ; Julianne Socha, USEPA; Annette DeMaria, ECT Inc.**

From: **Meghan McGahey, ECT Inc.**

Subject: **Expected Sampling Event # Notification**

Date: **(Insert date here)**

Sampling Event #(insert event # here)

Anticipated event date: **DATE**

Anticipated event start time: **TIME**

Contact **Meghan McGahey** or **Annette DeMaria** for sample collection and event status

Phone: **(596) 465-2583**

Field crews will call with estimated sample delivery time and will call again when they are within 30 minutes of Paragon Laboratory.

A shipment tracking code will be emailed to Source Molecular as well as an electronic copy of the Chain of Custody that is shipped with the samples.

If you no longer wish to receive these notifications, please contact Meghan McGahey or email that request to mmcgahey@ectinc.com.

Sampling Event Bottle Order Form

Needed By: _____:_____

Company: ECT

Team Leader: 586-465-2583 Meghan McGahey _____

Today's Date: _____

For (circle one): Pick Up or Delivery, deliver to: 44315 N. Groesbeck Hwy, Clinton Township, MI 48036

Type of Bottle	Type of Analysis	Preservative	***Required Amount Per Sample Event*	Number of Bottles Requested to Laboratory
150 mL Plastic	Microbiological (E. *coli*)	none	*130 minimum*	130

** For Internal Reference Only

Please include _____ large coolers.

***(Please check for ECT coolers that you may have obtained)**

Sample Collection and Handling

Standard Operating Procedure

1.0 Scope and Application

This procedure covers the collection, storage, and transport of water samples collected as part of the TMDL Sampling Program. The procedures are designed to maintain the integrity of the samples from the time they are collected to analysis at the laboratories.

2.0 Method Summary

Microbiological water samples (E. *coli*) will be taken as manual grab samples and must be collected directly into approved laboratory containers.

Manual Water samples are distributed to specific laboratory bottles that have been prepared and sterilized for E. *coli* analysis. The bottles are sized to provide the sample volume required by Paragon and Source Molecular Laboratories to perform the specific analysis. The label on each bottle is completed to identify the date, site ID, and sample type.

The filled lab bottles are placed in coolers for transfer to the laboratories. A chain of custody (COC) form is completed for all the bottles contained in a single cooler. Each cooler and its COC form should have only those bottles that are being sent to a single lab. Custody of the samples is transferred to the laboratory upon arrival.

3.0 Safety, Restrictions and Limitations

3.1 Safety

Protective clothing including gloves needs to be worn by field crew members when handling the samples to protect them against possible contaminants in the sample or preservatives in some of the laboratory bottles. At a minimum, sampling field personnel should be equipped with eye protection and gloves at all times when handling samples. Extreme care should be taken to prevent the possibility of ingesting any sample, including touching the mouth with hands, which have been handling samples.

3.2 Restrictions

The lab has varying volume requirements (minimum and optimum) for samples with which to perform various analyses. Water samples to be processed for *E. coli* will have as close to 100 mL as possible.

Specific bottle types and preservation techniques are required by the EPA to maintain the integrity of the sample prior to analysis.

3.3 Limitations

Bacteria samples using this method have a strict hold time of 6 hours for *E. coli* enumeration, therefore, samples for *E. coli* analysis will be delivered to Paragon within 5 hours of collection. Additionally, select duplicate samples will be shipped to Source Molecular Corporation via overnight courier service to be delivered the morning following collection. This will provide the samples to the laboratory for processing close to the 24 hour suggested hold time.

4.0 Sample Collection, Handling and Preservation

Sample collection is covered in section 6.1; Sample Handling is covered in section 6.3; and Sample Preservation is covered in section 6.4 (the only preservation for these samples is temperature maintained at/near 4°C).

5.0 Equipment and Material

a. High-powered lamps or flashlights
b. Field forms and log book
c. Keys to equipment storage
d. Safety equipment (personal and traffic)
e. Maps, routes and schedules
f. Field sampling plan with SOPs
g. Disposable latex gloves
h. Cooler with ice or ice packs
i. Appropriate standard data collection forms
j. Plastic bins for storage of laboratory bottles, by site
k. Chain of Custody forms
l. Zip lock bags
m. Multiple E. *coli* laboratory bottles (3 per site)

6.0 Procedures

Special Considerations

a. Samples to be analyzed for *E. coli* must be collected manually.

b. Samples to be analyzed for *E. coli* must be returned to the laboratory within the specified hold time.

6.1 Sample Collection

Samples will be taken manually by use of a sampling pole. The crew will divide the stream width, from bank to bank, into quarters. The stationing of the right and left quarter points and center will be determined. Left and right will always be oriented by looking upstream. A sterilized laboratory bottle will be placed into the sample water at each quarter and at approximately mid-depth with the sampler standing downstream of the sampling bottle to avoid contamination.

The task leader will identify the required collection sequence and the QA/QC samples to the sampling field staff.

6.2 Sample Handling

Sample handling procedures include:

a. Wear clean latex gloves during all sample handling procedures.

b. Keep the sample iced or refrigerated at all times following sample collection.

d. Do not expose the sample to light for an extended period of time.

c. Do not wash or rinse the laboratory bottles. They have been washed and prepared by the laboratory.

e. Do not overfill the laboratory bottles. Wear gloves and eye protection.

g. Do not attempt to fill more than one laboratory bottle at a time. A clear sequence of sample transfer from collection bottle/vessel to laboratory bottle must be maintained.

6.3 Sample Preservation

Sample bottles for *E. coli* analysis will be placed in cooler filled with ice as a preservative to maintain a temperature of 4°C. Sample bottles will be prevented from coming into direct contact with the ice, and exposure to light will be minimized.

6.4 Sample Labeling and Transfer

Each sample label will be completed by the sample team. Complete the sample label just prior to filling the bottle. Writing on the label is easier if the label is dry. The following labeling procedures should be:

a. The Analysis and Preservative sections of the sample bottle label should be completed prior to field sample collection.
b. Fill in the Sample Site section with the location designation.
c. Fill in the Sample ID section according to the following convention.

Sample identification will use the following Sample Numbering Scheme:

NN#SEV - d/b

Where:
NN# = Station Name/Location Identifier
DR = Detroit River
EC = Ecorse River/LeBlanc Drain
# = Site number

S = Sample (A, B, C, …) Always beginning with "A" on the left bank when facing upstream.
EV = Number of Event (01, 02, 03…22)
d = Field duplicate sample (this is to be left blank if the sample is not a duplicate sample)
b = Field blank sample (this is to be left blank if the sample is not a blank sample)

Example #1: DR8C06
This sample is from the Detroit River, collected from cross section #8. It is collected during the sixth week of sampling, and is the third sample collected from the left side of the river, when facing upstream.

Example #2: DR8C06 - d
This sample is from the Detroit River, collected from cross section #8. It is a duplicate of the sample collected during the sixth week of sampling, the third sample collected from the left side of the river, when looking upstream (duplicate of Example #1).

Example #3: EC5A02 – b
This sample is a blank sample, designated as being collected during week 2 on Ecorse River at site 5.

d. Fill in the Sample Date, Time, and the initials of the sampler in Sampled By.

e. Store the filled laboratory bottles in either a refrigerator or an ice chest. Group the filled bottles by the laboratory where the bottles will be sent. The project lab coordinator will define which labs to send the samples prior to the event.

f. Complete a chain of custody form. All samples listed on a single chain of custody form must be either in one cooler, or in one specific place in a refrigerator (such as the top shelf). Keep the pink copy of the form with the task leader. Place the original and yellow copy in a zip-loc bag in the ice chest with the samples, or in the refrigerator.

g. Transport the samples to the appropriate lab. Sign-off on the chain of custody is not required prior to transport to the laboratory. At the time of transfer, a sampling team member will sign over custody to the laboratory representative. The sampling team member should retain the yellow copy of the chain of custody form, after sign off by the laboratory. This copy should then be given to the task manager for review. The task manager will then forward a copy of the completed Chain of Custody to the Data and Laboratory Coordinator.

7.0 QA/QC

On-site QA/QC will be the responsibility of the field team manager. It shall be his or her responsibility to ensure that all field staff are trained and adequately

supervised in terms of sample handling procedures. It shall also be the coordinator's responsibility to ensure that all QA/QC samples are collected per the field sampling manual. Bottles will be stored at the central staging facility in defined bottle bins. Additional bottles for QA/QC samples will also be stored at this location.

Additional samples are required as the means to determine if contamination of the samples occurs due to improper handling. Four further quality assurance reviews of field procedures will occur based on the results of QA/QC analytical results. Implications of the field blanks and concerns regarding the accuracy of split samples will be disseminated to the field crews via the event coordinator.

The QA/QC samples that must be collected by the field crews include field duplicate samples and field blanks.

a. Field Blanks
   Field blanks are created by filling laboratory bottles with DI water in the same manner as the sample is collected and transferred to the laboratory bottle. The purpose of a field blank is to determine if field contamination may be affecting the analytical sample results.

   Field blanks will utilize the same sample volume and will be sent to the same production laboratory as receives the standard sample.

   For manual sample field blanks, perform the following:
   - Fill a clean laboratory bottle with DI water.
   - Cap and label.

b. Field Duplicates
   The field duplicates are collected to determine the variability at sampling sites. Field duplicate samples must be generated for all parameters.

   For manual sample field duplicates, perform the following:
   - Identify a location during each event where a duplicate will be collected.
   - Note location on the field form; it should vary from event to event.
   - Use a sterile collection bottle large enough to collect both the sample and the duplicate sample at the same time.

8.0 Computations, Documentation and Reporting

All field activities are recorded on the Sample Collection Data Sheets. The samples sent to the laboratories are documented on the COC forms. An example of the Sample Collection Data Sheet is attached.

9.0 Reference

None

TMDL Project **Event #_____**
Sample Collection Data Sheet

Crew: _____ **Date:** _____ **Time Started**: _____ **Time Ended**: _____

Weather conditions: ____ Sunny ____ Partly Cloudy/Sunny ____ Cloudy Temperature: _____

What light is available? ___ Sunlight ___ Cloudy ___ Daylight ___ High Powered Flashlight

Site ID	Flow (y/n)	Sample Time	E. *coli* Left ¼	E. *coli* Center	E. *coli* Right ¼	Water Color?	Same Color Across Width? (y/n)	Debris? (y/n)	Blank/DUP (Every 10th Sample)
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Were there problems with collecting any of the samples? ____ yes ____ no

Describe any problems:
_____

Field Lead Signature: _____ **Date:** _____

Example weekly data reporting format

** units in cfu/100mL*

DEQ Web Site ID	Station ID	Sample	Date Results	Date Dup	Date Results	Date Dup	Date Results	Date Dup	Date Results	Date Dup	Date Results	Date Dup	Date Results	Date Dup	30-day Geomean
	DR0	A	40		23		53		48		25		25		
		B	34		3,453		2,424		6,666		242		24,234		
		C	453		3,453		343,242		666		3,424		24,234		
		D	43,345		17		38		30		342		21		
		E	54		22	23	42		47		28	20	28	20	
Daily Geomean			270		159		588		197		182		387		266
	DR1	A	8		2		1		5		4		4		
		B	54		5		5		64		45		4,444		
		C	453		66		5		456		54		45		
		D	7		1		3		2		4		4		
		E	5	5	1		3		6	3	3		3		
Daily Geomean			23		4		3		18		10		25		10
	DR2	A	6		55		555		8		234		3		
		B	345		555		55		2,342		25,525		2,342		
		C	345		234,234		23,424		555		23		234		
		D	53		23,423		3,424,332		23,423		234		234	233	
		E	3		555		55		555		2,354		4		
		F	5		555		5,555	5	555		25		2		
Daily Geomean			29		1,929		3,013		649		352		38		337
	EC0	A	7		2		3		6		1		1		
	EC1	A	135		61		293		149		134		333		
		B	126		75		243		138		109		109		
		C	129	109	72		237		147		109	110	109	110	
Daily Geomean			130		69		256		145		117		158		135
	EC2	A	7		2		7		9		8		8		
		B	4		3		8		8		11		11		
		C	6		2		6		8	7	11		11		
Daily Geomean			6		2		7		8		10		10		6
	EC8	A	10		2		2		2		13		13		

= Precipitation occurred on the sample collection day and/or the day before.

= 30-day geometric mean > 130 cfu/100mL or daily geometric mean > 300 cfu/100mL

APPENDIX C

Paragon Laboratories
Quality Assurance Documentation

Preparation of Standard Methods / Plate Count Agar for Total Plate Count Analysis using Pour and Spread Plate Methods (PCA Agar Prep)

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microscopy, Media, Prep
Related Documents:	SOP-A0174, SOP-A0204
Applicable Programs:	Drinking Water, Wastewater
Regulatory References:	40*CFR*141, 40*CFR*136

1.2 This method is applicable to the preparation of Standard Methods / Plate Count Agar used for enumerating aerobic bacteria in water, wastewater, foods, and dairy products. This medium is also recommended as a general plating medium for determining bacterial populations.

2.0 SAFETY

2.1 For laboratory use only.

2.2 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.3 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

2.4 Follow proper, established procedures in handling and disposing of infectious materials.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flask - 1 L borosilicate glass

3.2 Sterile pipettes

3.3 Weighing boats

3.4 Petri plates - 100 x 15 mm

3.5 Refrigerator, maintained at 1-5°C

3.6 pH meter, capable of measuring pH to 0.01 SU

3.7 Balance, capable of measuring to 0.01 g

3.8 Hotplate

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3.9 Graduated cylinders - 100 mL, 500 mL, and 1000 mL

3.10 Incubator maintained at 44-46°C for tempering agar

3.11 Aluminum foil

3.12 Screw cap tubes

3.13 Autoclave or sterilizer capable of reaching 121°C at 15 lbs pressure

4.0 REAGENTS AND STANDARDS

4.1 Standard Methods / Plate Count Agar

4.2 Reagent-grade de-ionized (D.I.) water

4.3 *E. coli* pure culture

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 Seal dehydrated medium tightly, very hygroscopic.

5.2 The media, once made, is stored at 1-5°C and is good in loose lidded plated for two weeks and in screw cap tubes for two months.

5.3 The expiration date applies to the product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of Standard Methods / Plate Count Agar for performance (i.e., growth) with a known culture (*E. coli*).

6.2 Place one or more Standard Methods Agar / Plate Count Agar plates, from each batch, in the incubator for 24 hours at 35°C. Absence of growth indicates sterility of the plates.

6.3 Check the pH on each batch of agar after tempering to 44-46°C. The pH should be 7.0±0.2 SU. If the pH is out of range the medium must be discarded and re-made.

7.0 PROCEDURE

7.1 Suspend 23.5 grams in 1 liter D.I. water. Mix thoroughly.

7.2 Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

7.3 Autoclave at 121°C for 15 minutes. Temper in an incubator maintained at 44-46°C.

7.4 Measure the final pH of the agar after tempering to 44-46°C. If the pH is outside of 7.0±0.2 SU, discard the medium and re-make (refer to 6.3).

7.5 Test samples of the finished product for performance using stable, typical control cultures.

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7.6 Place solidified plates into Petri dish sleeve and seal tightly with tape or dispense into tightly capped screw cap tubes. Place plates in 1-5°C for storage for up to two weeks, or up to three months for screw cap tubes, before use.

8.0 REFERENCES

8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACW-240	M-00270-2001.doc	M-00270-2001	2001
1	Updated	ACW-240	M-00270-2001.doc	M-00270-2001	02/07/02
2	Reformatted	ACB-240	SOP-N0018-R2.doc	N0018	06/07/05
3	Clarified/Corrected 6.3 & 7.4	JMS-225	SOP-N0018-R3.doc	N0018	02/21/07
4					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Signed by: jcparmentier
Full Name: John Parmentier
Employee ID: 210
Signed from: JCPDK : PARAGON : jcparmentier
Signed on: 2007-02-22 11:23:52 GMT -05:00
Server Date/Time: 2007-02-22 11:23:55 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Signed by: jspurr
Full Name: John Spurr
Employee ID: 225
Signed from: ENVIROMGR : PARAGON : jspurr
Signed on: 2007-02-20 18:01:08 GMT -05:00
Server Date/Time: 2007-02-20 18:01:22 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Preparation of Tryptic Soy Broth / Trypticase Soy Broth for Quality Control of Dilution/Rinse Water, Sample Bottles, and as a Broth for Growing Pure Cultures (TSB Prep)

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microscopy, Media, Prep
Related Documents:	SOP-A0174, SOP-A0204
Applicable Programs:	Drinking Water, Wastewater
Regulatory References:	40*CFR*141, 40*CFR*136

1.2 This method is applicable to the preparation of Tryptic Soy Broth, a general purpose medium used in qualitative procedures for the cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens. The medium is used as a single strength preparation for growing pure cultures and checking the sterility of sample bottles. Use the medium in a double strength concentration when checking the sterility of dilution / rinse water.

2.0 SAFETY

2.1 For laboratory use only.

2.2 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.3 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

2.4 Follow proper, established procedures in handling and disposing of infectious materials.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flask (1 L borosilicate glass)

3.2 Sterile pipettes

3.3 Weighing boats

3.4 Screw cap tubes

3.5 Refrigerator (maintained at 1-5°C)

3.6 pH meter, capable of measuring pH to 0.01 SU

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3.7 Balance, capable of measuring to 0.01g

3.8 Hotplate

3.9 Graduated cylinders (100 mL, 500 mL, and 1000 mL)

3.10 Aluminum foil

3.11 Screw cap bottles

3.12 Autoclave or sterilizer capable of reaching 121°C at 15 lbs pressure

4.0 REAGENTS AND STANDARDS

4.1 Tryptic Soy Broth / Trypticase Soy Broth

4.2 Reagent-grade de-ionized water

4.3 *E. coli* pure culture

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 Seal dehydrated medium tightly, very hygroscopic.

5.2 The media, once made, is stored at 1-5°C and is good in loose lidded plated for two weeks and in screw cap tubes for two months.

5.3 The expiration date applies to the product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of Tryptic Soy Broth / Trypticase Soy Broth for performance (i.e., growth) with a known culture (*E. coli*).

6.2 Place one or more Tryptic Soy Broth / Trypticase Soy Broth tubes, from each batch, in the incubator for 24 hours at 35°C. Absence of growth indicates sterility of the plates.

6.3 Check the pH of each batch of broth. The pH should be 7.3±0.2 SU. If the pH is out of range the medium must be discarded and re-made.

7.0 PROCEDURE

7.1 TRYPTIC SOY BROTH / TRYPTICASE SOY BROTH – SINGLE STRENGTH

7.1.1 Suspend 30 grams in 1 liter de-ionized or distilled water. Mix thoroughly.

7.1.2 Heat with frequent agitation to completely dissolve the powder.

7.1.3 Dispense dissolved medium into screw cap tubes or bottles. DO NOT TIGHTEN DOWN CAPS.

7.1.4 Autoclave at 121°C for 15 minutes.

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7.1.5 Measure the final pH of the broth. If the pH is outside of 7.3±0.2 SU, discard the medium and re-make (refer to 6.3).

7.1.6 Test samples of the finished product for performance using stable, typical control cultures.

7.1.7 Place tightly capped screw cap tubes or bottles in 1-5°C for storage for up to three months.

7.2 TRYPTIC SOY BROTH / TRYPTICASE SOY BROTH – DOUBLE STRENGTH

7.2.1 Suspend 60 grams in 1 liter de-ionized or distilled water. Mix thoroughly.

7.2.2 Heat with frequent agitation to completely dissolve the powder.

7.2.3 Dispense dissolved medium into screw cap tubes or bottles. DO NOT TIGHTEN DOWN CAPS.

7.2.4 Autoclave at 121°C for 15 minutes.

7.2.5 Measure the final pH of the broth. If the pH is outside of 7.3±0.2 SU, discard the medium and re-make (refer to 6.3).

7.2.6 Test samples of the finished product for performance using stable, typical control cultures.

7.2.7 Place tightly capped screw cap tubes or bottles in 1-5°C for storage for up to three months.

8.0 REFERENCES

8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACW-240	M-00390-2001.doc	M-00390-2001	2001
1	Updated	ACW-240	M-00390-2001.doc	M-00390-2001	02/07/02
2	Reformatted	ACB-240	SOP-N0019-R2.doc	N0019	06/07/05
3	Clarified/Corrected 6.3, 7.1.5, & 7.2.5	JMS-225	SOP-N0019-R3.doc	N0019	02/21/07
4					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Full Name: John Parmentier
Employee ID: 210
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Server Date/Time: 2007-02-22 11:27:11 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
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Signed by: jspurr
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Signed on: 2007-02-20 17:58:41 GMT -05:00
Server Date/Time: 2007-02-20 17:58:56 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Nutrient Agar Preparation for Cultivating Bacteria and Enumerating Organisms in Water, Sewage, Feces, and Other Materials

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microbiology, E.coli
Related Documents:	SOP-A0204
Applicable Programs:	Wastewater, Surface Water, Drinking Water, Food
Regulatory References:	40*CFR*136, 40*CFR*141

1.2 This method is applicable to the preparation of Nutrient Agar used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, feces, and other materials.

2.0 SAFETY

2.1 For Laboratory use only.

2.2 Follow proper, established procedures in handling and disposing of infectious materials.

2.3 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.4 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flasks - 250 mL, 500 mL, and 1 L borosilicate glass

3.2 Sterile pipettes - 1 mL and 10 mL

3.3 Weighing boats

3.4 Small screw cap vials

3.5 Refrigerator, maintained at 1-5°C

3.6 pH meter, capable of measuring pH to 0.01 SU

3.7 Balance, capable of measuring to 0.01 g

3.8 Hotplate

3.9 Graduated cylinders - 100 mL, 500 mL, and 1000 mL

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3.10 Incubator maintained at 44-46°C for tempering agar

3.11 Aluminum foil

3.12 Autoclave or sterilizer capable of reaching 121°C at 15 lbs pressure

3.13 Test tube rack

3.14 Petri dishes - 58 x 13 mm or 100 x 15 mm

4.0 REAGANTS AND STANDARDS

4.1 Nutrient Agar

4.2 Reagent-grade de-ionized (D.I.) water

4.3 *E. coli* pure culture

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 All dehydrated media should be tightly sealed, very hygroscopic.

5.2 The media, once made, is stored at 1-5°C, and is good in screw cap tubes for two months (or in loose lidded plates for two weeks).

5.3 The expiration date applies to product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of Nutrient Agar for performance (i.e., growth) with a known culture (*E. coli*). *E. coli* will grow well on this medium.

6.2 Place one or more Nutrient Agar plates, from each batch, in the incubator for 24 hours at 35±0.5°C. Absence of growth indicates sterility of the plates.

6.3 Check the pH on each batch of Nutrient Agar after tempering to 44-46°C. The pH should be 6.8±0.2.

7.0 PROCEDURE

7.1 Suspend 23 grams of the powder in 1 liter of D.I. water. Mix thoroughly.

7.2 Boil for 1 minute to dissolve completely.

7.3 Dispense 5 mL portions into small screw cap tubes.

7.4 Autoclave at 121°C for 15 minutes.

7.5 Measure final pH. If pH is out of range the medium must be discarded and re-made.

7.6 Set tubes on a slant and allow to cool.

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7.7 Test samples of the finished product for performance using stable, typical control cultures.

8.0 REFERENCES

8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACB-240	SOP-N0060-R0.doc	N0060	02/21/07
1					
2					
3					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Signed by: jspurr
Full Name: John Spurr
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Reason Code: SOP Approved for Use
Placed Comment:

mTEC Agar and Urea Substrate Medium Preparation for Isolating, Differentiating, and Rapidly Enumerating Thermotolerant *Escherichia coli* from Water by Membrane Filtration and an *In Situ* Urease Test

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microbiology, E.coli
Related Documents:	SOP-A0204
Applicable Programs:	Wastewater, Surface Water, Drinking Water
Regulatory References:	40*CFR*136, 40*CFR*141

1.2 This method is applicable to the preparation of mTEC agar, used for isolating, differentiating, and rapidly enumerating thermotolerant *E. coli* from water by membrane filtration and an *in situ* urease test.

1.3 mTEC is an acronym for "membrane Thermotolerant *E. coli*." *E. coli* is widely used as an indicator of fecal pollution in water, and there are many procedures for enumerating *E. coli* based on its ability to grow at elevated temperatures and produce indole from tryptophan

1.4 mTEC Agar and urea substrate are recommended for use in the detection of *E. coli* when evaluating the microbiological quality of recreational waters.

2.0 SAFETY

2.1 For laboratory use only.

2.2 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.3 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

2.4 Follow proper, established procedures in handling and disposing of infectious materials.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flasks - 250 mL, 500 mL, and 1 L borosilicate glass

3.2 Weighing boats

3.3 Petri plates - 58 x 13 mm or 60 x 15 mm

3.4 Refrigerator, maintained at 1-5°C

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3.5 pH meter, capable of measuring pH to 0.01 SU

3.6 Balance, capable of measuring to 0.01 g

3.7 Hotplate

3.8 Graduated cylinders - 100 mL, 500 mL, and 1000 mL

3.9 Incubator maintained at 44-46°C for tempering agar

3.10 Aluminum foil

3.11 Autoclave or sterilizer, capable of reaching 121°C at 15 lbs pressure

4.0 REAGENTS AND STANDARDS

4.1 mTEC Agar

4.2 Reagent-grade de-ionized (D.I.) water

4.3 UREA SUBSTRATE MEDIUM

4.3.1 Urea

4.3.2 Phenol Red

4.3.3 Reagent-grade de-ionized (D.I.) water

4.3.4 1 N HCl

4.4 *E. coli* pure culture and *S. aureus* pure culture

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 Seal dehydrated medium tightly; it is very hygroscopic.

5.2 The media, once made, is stored at 1-5°C, and is good in loose lidded plates for two weeks (or in screw cap tubes for two months).

5.3 The expiration date applies to the product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of mTEC agar for performance (i.e., correct enzyme reactions) with known cultures (*E. coli* and *S. aureus*). After performing the urease test, *E. coli* will produce yellow, yellow-green, or yellow-brown colonies. The *S. aureus* culture will not produce yellow, yellow-green, or yellow-brown colonies after performing the urease test.

6.2 Place one or more mTEC agar plates, from each batch, in the incubator for 24 hours at 35±0.5°C. Absence of growth indicates sterility of the plates.

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6.3 Check the pH on each batch of mTEC Agar after tempering to 44-46°C. The pH should be 7.3±0.2. If the pH is out of range the medium must be discarded and re-made.

7.0 PROCEDURE

7.1 mTEC AGAR

7.1.1 Suspend 45.3g of the powder in 1 L of D.I. water.

7.1.2 Mix thoroughly.

7.1.3 Autoclave at 121°C for 15 minutes and cool in an incubator maintained at 44-46°C.

7.1.4 Dispense approximately 5 mL portions into 58 x 13 mm plates and allow to solidify.

7.1.5 Test samples of the finished product for pH, and performance using stable, typical control cultures (refer to 6.0).

7.1.6 Place solidified plates into petri dish sleeve and seal tightly with tape. Place plates in 1-5°C for storage for up to two weeks before use.

7.2 UREA SUBSTRATE MEDIUM

7.2.1 Prepare Urea Substrate by combining 2 g urea and 0.01 g (10 mg) phenol red in 100 ml of D.I. water.

7.2.2 Adjust the pH of the Urea Substrate Medium to 3-4 with 1 N HCl.

7.2.3 Store at 2-8°C, and use within 1 week.

8.0 REFERENCES

8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACB-240	SOP-N0059-R0.doc	N0059	02/21/07
1					
2					
3					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Reason Code: SOP Approved for Use
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Full Name: John Spurr
Employee ID: 225
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Signed on: 2007-02-20 17:56:21 GMT -05:00
Server Date/Time: 2007-02-20 17:56:31 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

EC Broth Preparation for Fecal Coliform Confirmation and for the Detection of Coliform Bacteria and of E. *coli* at an Elevated Temperature

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microbiology, Coliform, E.coli
Related Documents:	SOP-A0172, SOP-A0204
Applicable Programs:	Drinking Water, Wastewater
Regulatory References:	40*CFR*141, 40*CFR*136

1.2 This method is applicable to the preparation of EC Broth used for the detection of coliform organisms in water and wastewater at 35°C and for *E. coli* at an elevated temperature (44.5°C or 45.5°C). EC Broth is also used for the confirmation of fecal coliforms used in conjunction with Lauryl Tryptose Broth.

2.0 SAFETY

2.1 For laboratory use only.

2.2 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.3 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

2.4 Follow proper, established procedures in handling and disposing of infectious materials.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flask - 1 L borosilicate glass

3.2 Sterile pipettes

3.3 Weighing boats

3.4 Screw cap tubes

3.5 Refrigerator, maintained at 1-5°C

3.6 pH meter, capable of measuring pH to 0.01 SU

3.7 Balance, capable of measuring to 0.01 g

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3.8 Hotplate

3.9 Graduated cylinders - 100 mL, 500 mL, and 1000 mL

3.10 Aluminum foil

3.11 Autoclave or sterilizer capable of reaching 121°C at 15 lbs pressure

3.12 Fermentation or Durham tubes

3.13 Stir bar

4.0 REAGENTS AND STANDARDS

4.1 EC Broth

4.2 Reagent-grade de-ionized water

4.3 *E. coli* pure culture, *K. pneumoniae* pure culture, and *S. aureus* pure culture

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 Seal dehydrated medium tightly, very hygroscopic.

5.2 The media, once made, is stored at 1-5°C and is good in loose lidded plated for two weeks and in screw cap tubes for three months.

5.3 The expiration date applies to the product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of EC Broth for performance (i.e., growth) with known cultures. *E. coli* will produce gas which will be captured inside the fermentation tube at 44.5±0.2°C. *S. aureus* and *K. pneumoniae* will not produce gas 44.5±0.2°C.

6.2 Place one or more EC Broth tubes, from each batch, in the water bath for 24 hours at 44.5±0.2°C. Absence of growth indicates sterility of the tubes.

6.3 Check the pH on each batch of broth. The pH should be 6.9±0.2 SU. If the pH is out of range the medium must be discarded and re-made.

7.0 PROCEDURE

7.1 EC BROTH

7.1.1 Dissolve 37 grams of EC Broth powder in 1 liter de-ionized or distilled water. Mix thoroughly.

7.1.2 Warm slightly to completely dissolve the powder.

7.1.3 Dispense into tubes containing inverted fermentation vials.

7.1.4 Autoclave at 121°C for 15 minutes. Cool the broth as quickly as possible.

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7.1.5 Measure the final pH of the broth. If the pH is outside of 6.9±0.2 SU, discard the medium and re-make (refer to 6.3).

7.1.6 Test samples of the finished product for performance using stable, typical control cultures.

7.1.7 Place screw cap tubes in 1-5°C for storage for up to three months.

8.0 REFERENCES

8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACW-240	M-00065-2002.doc	M-00065-2002	03/04/02
1	Reformatted	ACB-240	SOP-N0022-R1.doc	N0022	07/22/05
2	Clarified/Corrected 6.3 & 7.1.5	JMS-225	SOP-N0022-R2.doc	N0022	02/21/07
3					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Authorization Mode: Database Login
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Full Name: John Spurr
Employee ID: 225
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Signed on: 2007-02-20 17:53:23 GMT -05:00
Server Date/Time: 2007-02-20 17:53:39 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Simmons Citrate Agar Preparation for the Differentiation of Gram-Negative Bacteria

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microbiology, E.coli
Related Documents:	SOP-A0204
Applicable Programs:	Wastewater, Surface Water, Drinking Water
Regulatory References:	40*CFR*136, 40*CFR*141

1.2 This method is applicable to the preparation of Simmons Citrate Agar, used for the differentiation of gram-negative bacteria on the basis of citrate utilization.

2.0 SAFETY

2.1 For laboratory use only.

2.2 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.3 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

2.4 Follow proper, established procedures in handling and disposing of infectious materials.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flasks - 250 mL, 500 mL, and 1 L borosilicate glass

3.2 Sterile pipettes

3.3 Weighing boats

3.4 Refrigerator, maintained at 1-5°C

3.5 Small screw cap test tubes

3.6 pH meter, capable of measuring pH to 0.01 SU

3.7 Balance, capable of measuring to 0.01 g

3.8 Hotplate

3.9 Graduated cylinders - 100 mL, 500 mL, and 1000 mL

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3.10 Incubator maintained at 44-46°C for tempering agar

3.11 Autoclave or sterilizer capable of reaching 121°C at 15 lbs pressure

4.0 REAGENTS AND STANDARDS

4.1 Simmons Citrate Agar

4.2 Reagent-grade de-ionized (D.I.) water

4.3 *E. coli* pure culture and *K. pneumoniae* pure culture

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 Seal dehydrated medium tightly, very hygroscopic.

5.2 The media, once made, is stored at 1-5°C, and is good in screw cap tubes for two months (or in loose lidded plates for two weeks).

5.3 The expiration date applies to the product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of Simmons Citrate agar for performance (i.e. correct enzyme reactions) with known cultures (*E. coli* and *K. pneumoniae*). *E. coli* produces no change in color (medium remains dark green, citrate is not utilized by organism). *K. pneumoniae* produces an intense blue color throughout the medium (organism utilizes citrate).

6.2 Place one or more Simmons Citrate agar tubes, from each batch, in the incubator for 24 hours at 35±0.5°C. Absence of growth indicates sterility of the tubes.

6.3 Check the pH on each batch of Simmons Citrate Agar after tempering to 44-46°C. The pH should be 6.9±0.2 SU. If the pH is out of range the medium must be discarded and re-made.

7.0 PROCEDURE

7.1 Suspend 24.2 g of the powder in 1 L of D.I. water. Mix thoroughly.

7.2 Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

7.3 Dispense 4 mL into small screw-cap tubes and autoclave at 121°C for 15 minutes.

7.4 Allow to cool in a slanted position for use as slants.

7.5 Store solidified tubes at 1-5°C for up to two months.

7.6 Test samples of the finished product for pH and for performance using stable, typical control cultures.

8.0 REFERENCES

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8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACB-240	SOP-N0061-R0.doc	N0061	02/21/07
1					
2					
3					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Reason Code: SOP Approved for Use
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Full Name: John Spurr
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Signed on: 2007-02-20 17:51:05 GMT -05:00
Server Date/Time: 2007-02-20 17:51:18 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Tryptone 1%; Tryptophane Broth Preparation for the Elaboration of Indole by Bacteria

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microbiology, E.coli
Related Documents:	SOP-A0204
Applicable Programs:	Wastewater, Surface Water, Drinking Water
Regulatory References:	40*CFR*136, 40*CFR*141

1.2 This method is applicable to the preparation of Tryptone 1%; Tryptophane Broth for the elaboration of indole by bacteria.

1.3 Bacto Tryptone is a pancreatic digest of casein. Casein is the main milk protein and a rich source of the amino acid nitrogen.

2.0 SAFETY

2.1 For laboratory use only.

2.2 IRRITANT

Irritating to eyes, respiratory system, and skin. Do not breathe dust.

2.3 FIRST AID

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this SOP or product label.

2.4 Follow proper, established procedures in handling and disposing of infectious materials.

3.0 EQUIPMENT AND SUPPLIES

3.1 Flasks - 250 mL, 500 mL, and 1 L borosilicate glass

3.2 Sterile pipettes

3.3 Weighing boats

3.5 Refrigerator, maintained at 1-5°C

3.8 Small screw cap test tubes

3.9 pH meter, capable of measuring pH to 0.01 SU

3.10 Balance, capable of measuring to 0.01 g

3.11 Hotplate

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3.12 Graduated cylinders - 100 mL, 500 mL, and 1000 mL

3.13 Incubator maintained at 44-46°C for tempering agar

3.14 Autoclave or sterilizer capable of reaching 121°C at 15 lbs pressure

4.0 REAGENTS AND STANDARDS

4.1 Tryptone or Trypticase Peptone

4.2 Reagent-grade de-ionized (D.I.) water

4.3 *E. coli* pure culture and *S. aureus* pure culture

4.4 Kovacs Indole Reagent

5.0 MEDIUM PRESERVATION AND HANDLING

5.1 Seal dehydrated medium tightly; it is very hygroscopic.

5.2 The media, once made, is stored at 1-5°C, and is good in screw cap tubes for two months (or in loose lidded plates for two weeks).

5.3 The expiration date applies to the product in its intact container when stored as directed. After the product is opened it is acceptable for one year from date of opening, unless product becomes discolored or clumps.

5.4 Do not use if product fails to meet specifications for identity and performance.

6.0 QUALITY CONTROL

6.1 Pretest each batch of Tryptone 1%; Tryptophane Broth for indole production (addition of Kovacs Indole Reagent will produce a deep red color in the alcohol layer of the broth if indole is produced) with known cultures (*E. coli* and *S. aureus*). *E. coli* will produce indole and turn the alcohol layer of the broth deep red. *S. aureus* will not produce indole and the broth will not turn deep red.

6.2 Place one or more Tryptone 1%; Tryptophane Broth tube from each batch, in the incubator for 24 hours at 35°C. Absence of growth indicates sterility of tubes.

6.3 Check the pH on each batch of Tryptone 1%; Tryptophane Broth after tempering to 44-46°C. The pH should be 7.2±0.2. If the pH is out of range the broth must be discarded and re-made.

7.0 PROCEDURE

7.1 Suspend 10g Tryptone or Trypticase Peptone in 1 L of D.I. water. Mix thoroughly.

7.2 Warm to dissolve completely.

7.3 Dispense in 5-ml volumes into tubes.

7.4 Autoclave at 121°C for 15 minutes and cool in an incubator maintained at 40-50°C.

7.5 Test samples of the finished product for pH and for performance using stable, typical control cultures.

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8.0 REFERENCES

8.1 Mary Jo Zimbro, B.S., MT and David A. Power, Ph.D. 2003. Difco & BBL Manual, Manual of Microbiological Culture Media. Becton, Dickinson and Company: Maryland.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACB-240	SOP-N0062-R0.doc	N0062	02/21/07
1					
2					
3					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Signed by: jcparmentier
Full Name: John Parmentier
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Signed from: JCPDK : PARAGON : jcparmentier
Signed on: 2007-02-22 11:24:36 GMT -05:00
Server Date/Time: 2007-02-22 11:24:38 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
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Signed on: 2007-02-20 17:50:04 GMT -05:00
Server Date/Time: 2007-02-20 17:50:24 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Escherichia coli (*E.* coli) in Water by Membrane Filtration and membrane-Thermotolerant *Escherichia coli* Agar (mTEC) using EPA 1103.1

1.0 SCOPE AND APPLICATION

1.1 REFERENCE INFORMATION

Discipline:	MIC, Microbiology, E. coli
Related Documents:	SOP-N0017, SOP-N0018, SOP-N0019, SOP-N0022, SOP-N0059, SOP-N0060, SOP-N0061, SOP-N0062
Applicable Programs:	Wastewater, Surface Water, Drinking Water
Regulatory References:	40CFR136, 40CFR141

1.2 BACKGROUND AND APPLICABILITY

1.2.1 This procedure is used for detecting and enumerating *Escherichia coli* (*E.* coli).

1.2.2 *E.* coli bacteria are a natural inhabitant only of the intestinal tract of warm-blooded animals. Its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens.

1.2.3 This procedure is used as a measure of recreational water quality. Epidemiological studies have led to the development of criteria, which can be used to promulgate recreational water standards based on established relationships between health effects and water quality. The significance of finding *E. coli* in recreational water samples is the direct relationship between the density of *E. coli* and the risk of gastrointestinal illness associated with swimming in the water (refer to reference 14.4).

1.2.4 This procedure can be applied to fresh, estuarine, and marine waters.

1.2.5 Since a wide range of sample volumes or dilutions can be analyzed by this procedure, a wide range of *E. coli* levels in water can be detected and enumerated.

2.0 SUMMARY OF METHOD

2.1 Using membrane filtration (MF) provides a direct count of *E.* coli bacteria in water based on the development of colonies on the surface of the membrane filter (refer to reference 14.5). A water sample is filtered through the membrane, which retains bacteria. After filtration, the membrane containing the bacterial cells is placed on a selective and differential medium, mTEC, incubated at 35±0.5°C for 2 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5±0.2°C for 22 hours. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 minutes, yellow, yellow-green, or yellow-brown colonies are counted with the aid of a fluorescent lamp and a magnifying lens. Verification procedures must be run on 1 sample per batch of 20 for confirmation as *E. coli*.

2.2 DEFINITIONS

2.2.1 In this procedure, *E. coli* are those bacteria which produce colonies that remain yellow, yellow-green, or yellow-brown on a filter pad saturated with urea substrate broth after primary culturing on mTEC medium.

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3.0 INTERFERENCES

Water samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or cause spreading of bacterial colonies, which could interfere with identification of target colonies.

4.0 SAFTEY

4.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilization equipment.

4.2 Mouth-pipetting is prohibited.

4.3 Autoclave all contaminated plates and materials after analysis.

5.0 EQUIPMENT AND SUPPLIES

5.1 Glass lens with magnification of 2-5x, or stereoscopic microscope.

5.2 A microscope lamp producing diffuse light from cool, white fluorescent lamps adjusted to give maximum color.

5.3 Hand tally or other electronic counting device.

5.4 Sterile T.D. (To Deliver) bacteriological or Mohr pipets, glass or plastic (1-mL and 10-mL volumes).

5.5 Graduated cylinders, 100-1000 mL.

5.6 Membrane filtration apparatus (filter base and funnel), glass, plastic or stainless steel, containing graduation marks in 50 mL intervals, able to hold a total volume of 300 mL. These are wrapped with aluminum foil or kraft paper and sterilized.

5.7 Vacuum source: line vacuum, electric vacuum pump, or aspirator.

5.8 Vacuum filter flask, usually 1 liter, with appropriate tubing. Filter manifolds to hold a number of filter bases are desirable, but optional.

5.9 Safety trap flask, placed between the filter flask and the vacuum source.

5.10 Forceps, straight (preferred) or curved, with smooth tips to permit easy handling of filters without damage.

5.11 Alcohol, 95% ethanol, in small wide-mouthed vials, for sterilizing forceps.

5.12 Alcohol burner.

5.13 Thermometer, checked against a National Institute of Science and Technology (NIST)-certified thermometer, or one traceable to an NIST thermometer.

5.14 Membrane Filters (MF), white, grid-marked, cellulose ester, 47-mm diameter, 0.45 µm ±0.02 µm pore size, pre-sterile.

5.15 Platinum inoculation loops, at least 3 mm diameter in suitable holders. (A platinum loop is

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required for the Cytochrome oxidase test in the verification procedure.)

5.16 Indelible ink marker for labeling plates.

5.17 Absorbent pads, sterile, 47-mm diameter.

5.18 Petri dishes, sterile, plastic, 9 x 50mm, with tight-fitting lids, or 15 x 60mm, glass or plastic, with loose-fitting lids; 15 x 100mm dishes may also be used.

5.19 Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions (if needed). Dilution bottles marked at 90 mL, or tubes marked at 9 mL may be used for 1:10 dilutions.

5.20 Flasks, borosilicate glass, screw-cap or covered with aluminum foil and sterilized, 250- to 2000-mL volume, for agar preparation.

5.21 Incubator maintained at 40°C to 50°C for tempering agar.

5.22 Incubator maintained at 35±0.5°C, with approximately 90% humidity if loose-lidded Petri dishes are used.

5.23 Incubator or water bath maintained at 44.5±0.2°C.

5.24 Test tubes, sterile, screw-cap, 20 x 150mm, borosilicate glass or plastic, with lids.

5.25 Test tubes, 10 x 75mm, borosilicate glass, with caps.

5.26 Test tubes screw-cap, borosilicate glass, 16 x 125mm or other appropriate size.

5.27 Whirl-Pak bags, or other appropriate zip-top-type bag.

5.28 Filter paper.

5.29 DILUTION WATER

Sterile phosphate-buffered dilution water, prepared in large volumes (e.g., 1 liter) for wetting membranes before addition of the sample and for rinsing the funnel after sample filtration or in 99-mL dilution blanks. Refer to SOP-N0017.

6.0 REAGENTS AND STANDARDS

6.1 PURITY OF REAGENTS

Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (refer to reference 14.7). The agar used in preparation of culture media must be of microbiological grade.

6.2 Use commercial culture media as a means of quality control.

6.3 PURITY OF WATER

Reagent-grade deionized (D.I.) water conforming to ASTM Specification D1193, Type II water or better (refer to reference 14.2).

6.4 BUFFERED DILUTION WATER

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Refer to SOP-N0017 for preparation.

6.5 mTEC AGAR & UREA SUBSTRATE MEDIUM

6.5.1 Use commercially available dehydrated media, where available.

6.5.2 Refer to SOP-N0059 for mTEC Agar and Urea Substrate Medium Preparation.

6.6 NUTRIENT AGAR

6.6.1 Use commercially available dehydrated media.

6.6.2 Refer to SOP-N0060 for Nutrient Agar Preparation.

6.7 TRYPTIC SOY BROTH

6.7.1 Use commercially available dehydrated media.

6.7.2 Refer to SOP-N0019 for Tryptic Soy Broth Preparation.

6.8 SIMMONS CITRATE AGAR

6.8.1 Use commercially available dehydrated media.

6.8.2 Refer to SOP-N0061 for Simmons Citrate Agar Preparation.

6.9 TRYPTONE 1%; TRYPTOPHANE BROTH

6.9.1 Use commercially available dehydrated media.

6.9.2 Refer to SOP-N0062 for Tryptone 1%; Tryptophane Broth Preparation.

6.10 EC BROTH

6.10.1 Use commercially available dehydrated media.

6.10.2 Refer to SOP-N0022 for EC Broth Preparation.

6.11 PLATE COUNT AGAR

6.11.1 Use commercially available dehydrated media.

6.11.2 Refer to SOP-N0018 for Plate Count Agar Preparation.

6.12 Cytochrome Oxidase Reagent; Oxidase Reagent

6.13 Kovacs Indole Reagent

7.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE

7.1 Collect samples in sterile poly sample containers with leak-proof lids.

7.2 If chlorine is suspected to be present in samples, sample container should contain sodium thiosulfate ($Na_2S_2O_3$).

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7.3 The reducing agent is necessary for the collection of water containing residual chlorine or other halogen, unless they contain broth for direct plating of sample. Sodium thiosulfate is a satisfactory de-chlorinating agent that neutralizes any residual halogen and prevents continuation of bactericidal action during sample transit. 1 mL, of a 10% $Na_2S_2O_3$ solution, per liter of water at the time of collection will be sufficient to neutralize halogen in the sample. Adherence to sample preservation procedures and holding time limits (see 7.11) are critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

7.4 When the sample is collected, leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Collect samples that are representative of the water being tested, flush or disinfect sample ports and use aseptic techniques to avoid sample contamination. Keep sampling bottle closed until it is to be filled. Remove cap, do not contaminate inner surface of cap or neck of bottle. Fill container without rinsing, replace cap immediately.

7.5 POTABLE WATER

If the sample is to be taken from a water distribution-system tap without attachments, select a tap that is supplying water from a service pipe directly connected with the main. Open tap fully and let water run to waste for 2 or 3 minutes, or for a time sufficient to permit clearing of the line. Reduce water flow to permit filling the bottle without splashing. If tap cleanliness is questionable, apply a solution of sodium hypochlorite (100 mg NaOCl / L) to faucet before sampling; let water run for a additional 2 or 3 minutes after treatment. Do not sample from leaking taps that allow water to flow over the outside of the tap. In sampling from a mixing faucet remove faucet attachments such as screen or splash guard, run hot water for 2 minutes, then cold for 2 to 3 minutes, and collect sample as indicated above. If sample is to be taken from a well fitted with a hand pump, pump water for 5 minutes before collecting sample. If the well has a mechanical pump collect sample directly from a tap on the discharge. If there is no pumping machinery, collect sample directly from the well by means of a sterilized bottle fitted with a weight at the base; take care to avoid contaminating the sample with any surface scum. In drinking water evaluation, collect samples of finished water from distribution sites selected to assure systematic coverage during each month. Choose distribution sites to include dead-end sections. Sample locations may be public sites, commercial establishments, private residences, and special sampling stations built into the distribution network.

7.6 RAW WATER SUPPLY

In collecting samples directly from a river, stream, lake, reservoir, spring, or shallow well, obtain samples representative of the water that is the source of supply to consumers. It is undesirable to take samples too near or too far from the point of draw off, or at a depth above or below the point of draw off.

7.7 SURFACE WATER

Select bacteriological sampling locations to include a baseline location upstream from the study area, industrial and municipal waste outfalls into the main stream area, tributaries except those with a flow less than 10% of the main stream, intake points for municipal or industrial water facilities, downstream samples based on stream flow time, and downstream recreational areas. Samples may be collected from a boat or a bridge near critical study points. Choose sampling frequency to be reflective of the stream or water body conditions. To monitor stream and lake water quality, establish sampling locations at critical sites. Sampling frequency will depend on the body of water in question.

7.8 BATHING BEACH

Sampling for recreational areas should reflect the water quality within the entire recreational zone.

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Include sites from upstream peripheral areas and locations adjacent to drains or natural contours that would discharge storm water collections or septic wastes. Collect samples in the swimming area from a uniform depth of ~1m. Consider sediment sampling of the water-beach interface because of exposure of young children at the waters edge. Relate sampling frequency to the peak bathing period. Collect daily samples during the recognized bathing season; minimum sampling includes Friday, Saturday, Sunday, and holidays.

7.9 SEDIMENT AND SLUDGE

Sediments may provide a stable index of the general quality of the overlying water, particularly where there is a great variability in its bacteriological quality. Sampling frequency in reservoirs and lakes may be related to seasonal changes in water temperature and storm water runoff. Bacteriological examination of sludges from water and wastewater treatment processes is desirable to determine the impact of their disposal into receiving water. Sludge monitoring may also indicate the effectiveness of wastewater treatment processes.

7.10 MANUAL SAMPLING

Take samples from a river, stream, lake, or reservoir by holding the bottle near its base in the hand and plunging it, neck downward, below the surface. Turn bottle neck until neck points slightly upward and mouth is directed toward the current (if there is no current, create one by pushing bottle forward horizontally in a direction away from the hand). When sampling from a boat, obtain samples from the upstream side of boat. If this is not possible, attach a weight to the base of the bottle and lower it into the water. Avoid contact with the bank or stream bed.

7.11 STORAGE TEMPERATURE AND HANDLING CONDITIONS

Ice or refrigerate water samples to a temperature of >0°C to ≤10°C during transit to the laboratory (refer to Table II of 40CFR136.3). Use insulated transportation containers (i.e. coolers) to assure proper maintenance of storage temperature. Do not allow sample bottles to become totally immersed in water from melted ice during transit or storage.

7.12 HOLDING TIME LIMITATIONS

Analyze samples as soon as possible after collection to avoid unpredictable changes. Do not hold samples longer than 6 hours between collection and initiation of analysis.

8.0 QUALITY CONTROL

8.1 Test new lots of membrane filters by placing a membrane on a plate of Plate Count Agar and incubating for 48 hours at 35±0.5°C. Check for growth.

8.2 FUNNEL CONTROL

Perform a sterility check on each funnel in use at the beginning and end of each filtration series (filtration series ends when 30 minutes or more elapse between sample filtrations). Before filtering samples, place one membrane filter on each funnel base in use, attach funnel, and filter 30mL sterile rinse water through funnel. Then place filter on an mTEC Agar plate. Repeat at end of filtration cycle after last samples are run and rinsed thoroughly with two 30mL portions of sterile rinse water. Invert plates and incubate the plate at 35±0.5°C for 2 hours. After a 2-hour incubation at 35±0.5°C, transfer the plates to a Whirl-Pak, or other appropriate zip-top type bag. Keep the plates inverted and put into an incubator or water bath maintained at 44.5±0.2°C for 22-24 hours. After 22-24 hours, remove the plates from the incubator or water bath. Place an absorbent pad in a new Petri dish or in the lid of the same Petri dish, and saturate the pad with Urea Substrate Medium. Aseptically transfer the membrane from mTEC Agar to the absorbent pad saturated with Urea Substrate Medium, and allow to sit at room temperature for 15-20

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minutes. Absence of growth, after incubation on the urea substrate at room temperature, indicates satisfactory rinsing and no cross contamination.

8.3 PHOSPHATE-BUFFERED DILUTION / RINSE WATER CONTROLS

Sterilize a dilution bottle containing 50-mL of dilution buffer (with each batch of dilution bottles and/or rinse buffer made). After the 50-mL bottle has cooled to room temperature, add 50-mL of Double Strength Tryptic Soy Broth and incubate for 48 hours at 35±0.5°C. Absence of growth indicates sterility of the dilution / rinse water.

8.4 AGAR CONTROLS

8.4.1 Pretest each batch of mTEC agar for performance (i.e., correct enzyme reactions) with known cultures (*E. coli* and a non-coliform), a blank, and for correct pH.

8.4.2 Pretest each batch of Nutrient Agar, Plate Count Agar, Tryptic Soy Broth (single and double strength), and Tryptone 1% for performance (*E. coli* and a blank) and for correct pH.

8.4.3 Pretest each batch of EC Broth for performance (i.e., gas formation) with known cultures (*E. coli* and a total coliform), a blank, and for correct pH.

8.4.4 Pretest each batch of Simmons Citrate Agar for performance (i.e., correct enzyme reactions) with known cultures (*E. coli,* and *Klebsiella pneumoniae*), a blank, and for correct pH.

8.5 Perform sterility control on each new lot number of pre-sterilized sample bottles using 25 mL of single strength Tryptic Soy Broth. Incubate for 48 hours at 35±0.5°C and observe for growth. Absence of growth indicates sterility of the bottles.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Check temperatures in incubators twice daily to ensure operation within stated limits.

9.2 Check thermometers at least annually against an NIST-certified thermometer or one traceable to NIST. Check mercury columns for breaks.

10.0 PROCEDURE

10.1 ROUTINE INITIAL PROCEDURE

10.1.1 Prepare mTEC agar and Urea Substrate Medium as described in 6.5.

10.1.2 Label the bottom of the mTEC agar plates with the sample number/identification and the volume of sample to be analyzed.

10.1.3 Using a sterilized forceps, place a sterile membrane filter, grid-side up, on the porous plate of the filter base. If you have difficulties in removing the separation papers from the filters due to static electricity, place a filter with the paper on top of the funnel base and turn on the vacuum. The separation paper will curl up, allowing easier removal.

10.1.4 Attach the funnel to the base of the filter unit, taking care not to damage or dislodge the filter. The membrane filter is now located between the funnel and the base.

10.1.5 Put approximately 30 mL of sterile dilution water in the bottom of the funnel.

10.1.6 Shake the sample container vigorously 25 times to distribute the bacteria uniformly, and measure the desired volume of sample or dilution into the funnel.

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10.1.7 Select sample volumes based on previous knowledge of the pollution level, to produce 20-80 *E. coli* colonies on the membranes. Sample volumes of 1-100 mL are normally tested at half-log intervals (e.g. 100, 30, 10, 3 mL).

10.1.8 Smaller sample sizes or sample dilutions can be used to minimize the interference of turbidity or for high bacterial densities. Multiple volumes of the same sample or sample dilutions may be filtered, and the results may be combined. Typically a 10X, 100X, and 10000X dilution will provide suitable sample results.

10.1.9 Filter the sample, and rinse the sides of the funnel with 2 (20-30 mL) portions of sterile buffered rinse water.

10.1.10 Remove the funnel from the base of the filter unit.

10.1.11 Holding the membrane filter at its edge with a flamed forceps, gently lift and place the filter grid-side up on the mTEC agar plate. Slide the filter onto the agar, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar. Reseat the membrane if non-wetted areas occur due to air bubbles.

10.1.12 Rinse the funnels 4-6 more times with 20-30 mL portions of sterile buffered rinse water.

10.1.13 Invert the agar Petri dish, and incubate the plate at 35 +/- 0.5°C for 2 hours. If loose-lidded plates are used for mTEC agar, the plates should be placed in a humid chamber.

10.1.14 After a 2-hour incubation at 35±0.5°C, transfer the plate(s) to a Whirl-Pak, or other appropriate zip-top-type bag. Keep the plate(s) inverted and put into an incubator or water bath maintained at 44.5±0.2°C for 22-24 hours.

10.1.15 After 22-24 hours, remove the plate(s) from the incubator or water bath. Place an absorbent pad in a new Petri dish or in the lid of the same Petri dish, and saturate the pad with Urea Substrate Medium. Aseptically transfer the membrane from mTEC Agar to the absorbent pad saturated with Urea Substrate Medium, and allow to sit at room temperature for 15-20 minutes.

10.1.16 After incubation on the urea substrate at room temperature, count and record the number of yellow, yellow-green, or yellow-brown colonies on the membrane filters, ideally containing 20-80 colonies.

10.2 VERIFICATION PROCEDURE

10.2.1 Yellow, yellow-green, or yellow-brown colonies from the Urease test can be verified as *E. coli*. One sample per 20 must be verified and confirmed as *E. coli*.

10.2.2 Using a sterile inoculation loop, transfer growth from the centers of at least 10 well-isolated colonies to Nutrient Agar slants and to Tryptic Soy Broth. Incubate the agar and broth cultures for 24 hours at 35±0.5°C.

10.2.3 After incubation, remove a loopful of growth from the Nutrient Agar slant with a platinum loop, and deposit it on the surface of a piece of filter paper that has been saturated with Cytochrome Oxidase Reagent. If the spot where the bacteria were deposited turns deep purple within 15 seconds, the test is positive.

10.2.4 Transfer growth from the Tryptic Soy Broth tube to Simmons Citrate Agar, Tryptone 1% Broth, and an EC Broth fermentation tube.

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10.2.4.1 Incubate the Simmons Citrate Agar and Tryptone 1% Broth for 48 hours at 35±0.5°C.

10.2.4.2 Incubate the EC Broth at 44.5±0.2°C in an incubator for 24 hours.

10.2.4.3 Add 0.5 mL of Kovacs Indole Reagent to the 48-hour Tryptone 1% Broth culture, and shake gently. A positive test for indole is indicated by a deep red color which develops in the alcohol layer on top of the broth.

10.2.5 *E. coli* is EC gas-positive, indole-positive, oxidase-negative, and does not utilize citrate (i.e. the medium remains green).

11.0 CALCULATIONS AND DATA ANALYSIS

11.1 Use the following general rules to calculate the *E. coli* per 100 mL of sample.

11.1.1 Select the membrane filter with an acceptable number of yellow, yellow-green, or yellow brown colonies (20-80) on the urea substrate.

11.1.2 If the total number of colonies on a filter are too-numerous-to-count (TNTC) or confluent, record the results as >80 x the dilution factor used. For example, if >80 colonies are counted on the 100X dilution, report as >8000 CFU/100mL.

11.1.3 Calculate the final values using the formula:

$$\textit{E. coli} \text{ / 100 mL} = \frac{\text{Number of } \textit{E. coli} \text{ colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

11.2 Refer to the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules.

11.3 Report results as CFU *E. coli* /100 mL sample.

12.0 METHOD PERFORMANCE

12.1 PERFORMANCE CHARACTERISTICS

12.1.1 Precision – The degree of agreement of repeated measurements of the same parameter expressed quantitatively as the standard deviation or as the 95% confidence limits of the mean computed from the results of a series of controlled determinations. The mTEC method precision was found to be fairly representative of what would be expected form the counts with a Poisson distribution (refer to reference 14.5).

12.1.2 Bias – The persistent positive or negative deviation of the average value of the method from the assumed or accepted true value. The bias of the mTEC method has been reported to be -2% of the true value (refer to reference 14.5).

12.1.3 Specificity – The ability of a method to select and/or distinguish the target bacteria under the test from other bacteria in the same water sample. The specificity characteristic of a method is usually reported as the percent of false positive and false negative results. The false positive rate reported for mTEC medium averaged 9% for marine and fresh water samples. Less than 1% of the *E. coli* colonies observed gave a false negative reaction (refer to reference 14.5).

12.1.4 Upper Counting Limit (UCL) – That colony count above which there is an unacceptable counting error. The error may be due to over crowding or antibiosis. The UCL for *E. coli* on mTEC medium has been reported as 80 colonies per filter (refer to reference 14.5).

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12.2 COLLABORATIVE STUDY DATA

12.2.1 A collaborative study was conducted among eleven volunteer laboratories, each with two analysts who independently tested local fresh and marine recreational waters and sewage treatment plant effluent samples, in duplicate. The data were reported to the USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, for statistical calculations.

12.2.2 The results of the study are shown in Figure 1 of method 1103.1, where S_o equals the pooled standard deviation among replicate counts from a single analyst for three groupings (counts less than 30, counts from 30 to 50, and counts greater than 50) and S_B equals the pooled standard deviation between means of duplicates from analysts in the same laboratory for the same groupings. The precision estimates from this study did not show any difference among the water types analyzed.

12.2.3 By linear regression, the precision of the method can be generalized as:

S_o = 0.028 count/100mL + 6.11 (dilution factor) and
S_B = 0.233 count/100mL + .082 (dilution factor)

$$\text{Where dilution factor} = \frac{100}{\text{Volume of Original Sample Filtered}}$$

12.2.4 Because of the instability of microbial populations in water samples, each laboratory analyzed its own sample series and no full measure of recovery of bias was possible. However, all laboratories analyzed a single surrogate sample prepared from a freeze-dried culture of *E. coli.* The mean count and the overall standard deviation of the counts (which includes the variability among laboratories for this standardized *E. coli* sample) were 31.6 colonies/membrane and 7.61 colonies/membrane, respectively.

13.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

13.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique, such as preparation of the smallest practical volumes of reagents, standards, and media or downsizing of the test units in a method. The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

13.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

13.3 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. All infectious wastes should be autoclaved before disposal.

13.4 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules.

13.5 Samples, reference materials, and equipment known or suspected to have viable *E. coli* attached or contained must be sterilized prior to disposal.

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13.6 Samples preserved with HCl to pH <2 are hazardous and must be neutralized before being disposed, or must be handled as hazardous waste.

13.7 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less Is Better: Laboratory Chemical Management for Waste Reduction," both available from the American Chemical Society's Department of Government Relations and Science Policy, 115 16th Street NW, Washington, DC 20036.

14.0 REFERENCES

14.1 USEPA. Office of Water. 2006. Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). EPA-821-R-06-010. Washington: USEPA, July.

14.2 American Society for Testing and Materials. Annual Book of ASTM Standards, Vol. 11.01. Philadelphia, PA 19103.

14.3 USEPA. Office of Research and Development (ORD). Bordner, R., J.A. Winter, and P.V. Scarpino (eds.). Microbiological Methods for Monitoring the Environment, Water, and Wastes. EPA-006/8-78-017. Washington: USEPA.

14.4 Cabelli, V.J., A.P. Dufour, M.A. Levin, L.J. McCabe, and P.W. Haberman. 1979. Relationship of Microbial Indicators to Health Effects at Marine Bathing Beaches. *Am. J. Public Health.* 69: 690-696.

14.5 Dufour, A.P., E.R. Strickland, V.J. Cabelli. 1981. Membrane Filter Method for Enumerating *Escherichia coli*. *Appl. Environ. Microbiol.* 41:1152-1158.

14.6 USEPA. Office of Science and Technology. 2000. Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli.* EPA/821/R-97/004. USEPA: Washington.

14.7 American Chemical Society (ACS). Reagent Chemicals: American Chemical Society Specifications. ACS: Washington. [For suggestions of the testing of reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, UK and the United States Pharmacopeia.]

14.8 USEPA. Environmental Monitoring and Support Laboratory (EMSL). 1985. Test Methods for *Escherichia coli* and enterococci in Water by the Membrane Filter Procedure. EPA-600/4-85/076. USEPA: Cincinnati.

Revision History

Rev	Description of Change	Originator	Source File	Source SOP	Effective Date
0	Initial Release	ACB-240	SOP-A0204-R0.doc	A0204	02/22/07
1					
2					
3					

Approvals

Primary Authority:	John C. Parmentier, Vice President (Name and Title)
Secondary Authority:	John M. Spurr, Manager - Process & Systems (Name and Title)

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Attachment 1
Procedural Checklist for
Escherichia coli (*E.* coli) in Water by Membrane Filtration and membrane-Thermotolerant *Escherichia coli* Agar (mTEC) using EPA 1103.1

1.
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7. [R E S E R V E D]
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11.
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14.

(For more detail refer to the full SOP.)

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Attachment 2
Quality Control (QC) Criteria Summary for *Escherichia coli* (*E.* coli) in Water by Membrane Filtration and membrane-Thermotolerant *Escherichia coli* Agar (mTEC) using EPA 1103.1

Calibration / Instrument Performance

QC Component	Clarifications	Frequency	Acceptance Criteria	Corrective Actions & Contingencies
Setup and/or Conditioning	n/a	n/a	n/a	n/a
Membrane Filter Checks	Test on Plate Count Agar	1 per new Lot No.	Zero colonies counted	Reject Lot No. if >0 colonies are counted (if any growth is noted)
Phosphate-Buffered Dilution/Rinse Water Sterility Checks	Test with double-strength Tryptic Soy Broth	Each batch of dilution bottles and/or rinse buffer	Broth remains clear (and colorless) with no precipitate	Recheck another bottle from the same batch to rule out contamination from air or pipet surface: --If acceptable, batch is OK; --If not acceptable, reject the batch, rewash the bottles, and reprep.
Agar Performance Checks (4)	Test with a known positive culture, with a known negative culture, as a blank, and for correct pH	1 of each of the 4 checks per new batch of each agar	Varies by agar and check (refer to each referenced SOP)	Reject agar batch if out of specifications (refer to each referenced SOP)
Other (specify)	n/a	n/a	n/a	n/a

Method & Sample Performance

QC Component	Clarifications	Frequency	Acceptance Criteria	Corrective Actions & Contingencies
MBs (Filter Funnel Sterility Checks)	Test Each Filter Funnel on mTEC Agar using Dilution/Rinse Water	At Beginning and End of Each Filtration Series (<30 minutes between filtrations)	Zero colonies counted	"Flag" all results prepared from specific funnel within preparation series if >0 colonies are counted (if any growth is noted)
LCS	n/a	n/a	n/a	n/a
MS / MSD	n/a	n/a	n/a	n/a
DUP	Test any submitted sample in duplicate	1 per 20 samples	(Not Specified)	Report, as requested per project
Other (specify)	n/a	n/a	n/a	n/a

Initial Demonstration of Capability (IDOC)

QC Component	Clarifications	Frequency	Acceptance Criteria	Corrective Actions & Contingencies
MB	True Negative Verification	Initial Training	Zero colonies counted	Troubleshoot with trainer (manager); redo
IPR	n/a	n/a	n/a	n/a
MDL	n/a	n/a	n/a	n/a
QC Known	ERA QC Sample	Initial Training	Within QC limits	Troubleshoot with trainer (manager); redo
Positive Sample Reproducibility	n/a	n/a	n/a	n/a
Other (specify)	n/a	n/a	n/a	n/a

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Signed by: jspurr
Full Name: John Spurr
Employee ID: 225
Signed from: ENVIROMGR : PARAGON : jspurr
Signed on: 2007-02-22 11:40:40 GMT -05:00
Server Date/Time: 2007-02-22 11:40:52 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
Placed Comment:

Signed by: jcparmentier
Full Name: John Parmentier
Employee ID: 210
Signed from: JCPDK : PARAGON : jcparmentier
Signed on: 2007-02-22 11:29:58 GMT -05:00
Server Date/Time: 2007-02-22 11:29:59 GMT -05:00
Authorization Mode: Database Login
Reason Code: SOP Approved for Use
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Paragon Laboratories, Inc.

CHAIN-OF-CUSTODY RECORD

12649 Richfield Court • Livonia, MI 48150 • (734) 462-3900 • Fax (734) 462-3911

Paragon Report No.

Page_____ of _____

Client Name	Logged By: Checked By:
Contact Person:	Sample Condition Upon Receipt: Acceptable Other (Specify below)
Mailing Address:	
City, State, Zip Code:	
Phone: Fax:	Volatiles Preserved: Yes No Metals ff: Yes No pres: Yes No
Client Job Name/No.:	Remarks:
Job Loc:	
Sampled By: P.O. No.:	ANALYSIS REQUESTED

Regulatory Requirements
- MERA TMDL's ☐
- RCRA ☐
- NPDES ☐
- Drinking Water ☐
- Other: _____

Turnaround Requirements
- Standard 5-7 DAY ☐
- 2 Day (RUSH) ☐
- 24 Hour (RUSH) ☐
- SPECIAL ☐

Matrix Key
- S = Soil
- W = Water
- O = Oil
- SL = Sludge
- A = Air
- X = Other

ITEM NO.	DATE TAKEN	TIME TAKEN	GRAB	COMP.	CLIENT SAMPLE ID	MATRIX	NUMBER OF CONTAINERS											PARAGON SAMPLE NO.	Possible Health Hazard

Trans. #	RELEASED BY	RECEIVED BY	DATE	TIME	Trans. #	RELEASED BY	RECEIVED BY	DATE	TIME
1)					3)				
2)					4)				

APPENDIX D

Source Molecular Corporation
Quality Assurance Documentation

SOURCE MOLECULAR CORPORATION
4989 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-268-8363, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Laboratory Quality Assurance / Quality Control Plan

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Laboratory Quality Assurance / Quality Control Plan

1. Quality Policy Statement and Commitments by Top Management

The directors of the Source Molecular Corporation are committed to upholding the highest degree of professionalism and expertise in all aspects of Environmental and Molecular Microbiology. The laboratory focuses on identification of microbial pathogens and indicators found in water and wastewater as well as in identification of potential sources of fecal contamination (Microbial Source Tracking) in environmental waters. The laboratory is committed to producing and reporting sound and verifiable data that can be used by water quality managers and policymakers as tools for remediation and risk assessment.

2. Organization and Management Structure

Director/Principal Investigator/QA Officer - Troy M. Scott, M.S., Ph.D., Environmental and Molecular Microbiology
Director/Principal Investigator – Jerzy Lukasik, M.S., Ph.D., Environmental Microbiology
Laboratory Manager – Joel Caren, M.S., Plant Molecular and Cell Biology
Director/Business Strategist - Thierry Sam Tamers, B.S., Finance

3. Relationship Between Management, Support Services, and Quality System

Dr. Troy M. Scott and Dr. Jerzy Lukasik serve as directors and principal investigators of the laboratory. Joel Caren is responsible for all bench activities and reports directly to Dr. Lukasik and Dr. Scott. Dr. Scott is also the laboratory QA Officer and is responsible for implementing and enforcing standard operating procedures as well as maintaining all calibration and monitoring logs. Thierry Tamers is in charge of administrative functions.

4. Job Descriptions of Key Staff

Troy M. Scott, Ph.D. – Laboratory Director/QA Officer – Oversees and validates final results. Implements and enforces all quality assurance/quality control measures. Supervises field collection and processing of samples, and directs the environmental parasitology and molecular biology divisions of the company.

Jerzy Lukasik, Ph.D. – Laboratory Director - Oversees and validates final results, prepares final reports, supervises analyses, serves as director of Research and Development, heads tissue cell culture laboratory, and directs the environmental virology laboratory.

Joel Caren, M.S. – Laboratory Manager - Supervises the environmental microbiology laboratory and oversees research and development of novel methods to detect pathogens in environmental samples.

Thierry Sam Tamers, B.S. – Manages the administrative and financial aspects of the company.

5. Identification of Approved Signatories for the Laboratory

Either Dr. Lukasik or Dr. Scott must approve all laboratory reports.

6. List of all Test Methods Under Which Testing is Being Performed

Standard Operating Procedures – All standard operating procedures (EPA methods, ICR, Standard Methods, ASTM) are available to all personnel in the SOP notebook or in reference manuals.

6.1 SM9215B (Heterotrophic Bacteria) Pour plate technique in accordance with the Safe Drinking Water Act and Standard Methods for the Examination of Water and Wastewater.

Summary of Method

The pour plate method is used for analysis of heterotrophic plate count (HPC) bacteria. This method can accommodate volumes of sample or dilutions of up to 2 mL. Plate count agar can be purchased from DIFCO as a dehydrated media. Briefly, water samples are added to the bottom of a sterile petri dish. Immediately following addition of sample, 16-20 mL of molten plate count agar (cooled to 45°C) is added to the plate and swirled in a figure 8 motion to disperse sample and media within the plate. USEPA's Surface Water Treatment Rule (40 CFR 141.94) dictates a 48 hour incubation at 35°C; however, the highest counts are typically obtained from a 5-7 day incubation at temperatures between 20 and 28 ^{o}C. Duplicate samples are often incubated at both temperatures to ensure accuracy of results.

6.1.1 Quality Control

Escherichia coli C-3000 (ATCC 15597) is used as a positive control for verification of media integrity. Colonies that grow within the media are considered as positive verification of heterotrophic plate count bacteria. A negative sample is performed by filtering sterile PBS and incubating along with positive control sample.

6.2 SM9222B (Total Coliform) Membrane Filtration Method in accordance with the Safe Drinking Water Act and Standard Methods for the Examination of Water and Wastewater.

Summary of Method

Total coliforms are analyzed by the membrane filter technique using m-Endo media. Media is prepared by dissolving 51 g of powdered m-Endo LES agar per liter deionized water and heated while stirring with a magnetic stir bar. Once media has boiled, 20 ml of 95% ethanol is added and the media is dispensed into plates. Agar plates are kept refrigerated and wrapped in aluminum foil to exclude light. Plates are kept for a maximum of two weeks. After water samples have been passed through membrane filters, each filter is placed on an m-endo media plate and

incubated, inverted, for 24 +/- 2 hours at 35° ± 0.5 C. Total coliform colonies are those which are pink to dark red with a metallic-green sheen. Light pink, blue, or white colonies are considered non-coliforms.

6.2.1 Quality Control

Escherichia coli C-3000 (ATCC 15597) is used as a positive control for verification of media integrity. Colonies that grow and exhibit dark red pigmentation and metallic sheen are considered as positive verification of total coliform bacteria. A negative sample is performed by filtering sterile PBS and incubating along with positive control sample.

6.3 SM9222D (Fecal Coliform Bacteria) Membrane Filtration Method in accordance with the Safe Drinking Water Act and Standard Methods for the Examination of Water and Wastewater.

Summary of Method

Fecal coliforms are analyzed by the membrane filter technique using membrane fecal coliform (mFC) media. mFC media is prepared by dissolving 52 g of dehydrated medium per liter of deionized water and heated while stirring with a magnetic stir bar. Suspension is boiled to dissolve for one minute, followed by the addition of 10 ml 1% rosalic acid in 0.2 N NaOH per liter of media. Liquifed media is then dispensed into plates, which can be kept refrigerated for up to 2 weeks. For analysis, after water samples have been passed through membrane filters, each filter is placed on an mFC media plate, plates are placed into whirl-pack bags with waterproof enclosures and incubated submerged in a water bath at 44.5 ± 0.2° C for 24 +/- 2 hours. Blue to blue-gray colonies are counted as fecal coliforms. Pink, cream, gray or other non-blue colored colonies are not considered fecal coliforms.

6.3.1 Quality Control

Escherichia coli C-3000 (ATCC 15597) is used as a positive control for verification of media integrity. Colonies that grow and exhibit dark blue pigmentation are considered as positive verification of Fecal coliform bacteria. A negative sample is performed by filtering sterile PBS and incubating along with positive control sample.

6.4 SM9230C (*Enterococcus spp.*) or EPA Method 1600 - Membrane Filtration Method in accordance with the EPA Safe Drinking Water Act and Standard Methods for the Examination of Water and Wastewater.

Summary of Method

One of two methods are used for enterococci analysis, either the mE or mEI methods. The mEI method is more commonly used and it involves membrane filtration with mEI agar plates. mEI agar is prepared by dissolving 71.2 g dehydrated mE agar (Difco) and 750 mg indoxyl β-D-glucoside per liter deionized water and autoclaving for 15 minutes at 121° C. Autoclaved media is cooled to 45-

50° C in a water bath, and to each liter of media is added 10 ml of a 24 mg/ml nalidixic acid solution and 0.2 ml of a 10% 2,3,5-triphenyltetrazolium chloride solution; both reagent supplements are dissolved in sterile deionized water. Media is dispensed into plates and allowed to solidify. Plates are stored wrapped in aluminum foil to exclude light and refrigerated. Plates are kept for a maximum of two weeks.

After water samples have been passed through membrane filters, filters are placed on mEI plates and incubated at 41° C for 24 +/- 2 hours. Enterococci colonies are small, gray colonies with a blue fringe. Only colonies with this appearance are counted as enterococci.

An alternate membrane filter procedure for enterococci uses mE media plates with a second incubation on Esculin iron agar (EIA) substrate plates. mE agar plates are prepared by dissolving 71.2 g dehydrated mE agar per liter deionized water and autoclaving for 15 minutes at 121° C. Autoclaved media is cooled to 45-50° C in a water bath, and to each liter is added 10 ml of a 24 mg/ml nalidixic acid solution and 0.2 ml of a 10% 2,3,5-triphenyltetrazolium chloride solution; both reagent supplements are dissolved in sterile deionized water. Media is dispensed into plates and allowed to solidify. EIA agar plates are prepared by dissolving 16.5 g dehydrated medium per liter deionized water. Media is sterilized by autoclaving for 15 minutes at 121° C. After dispensing media into petri dishes and allowing to solidify, plates are stored refrigerated for up to two weeks. With the mE procedure, membrane filters through which water samples have been passed are placed first on mE plates and incubated for 48 hours at 41 +/- 0.5° C. After 48 hours incubation, each filter is transferred to an EIA plate and incubated at 41° C for 20 minutes. Colonies which appear pink to red, with a black or reddish brown precipitate on the underside of the filter after EIA incubation are counted as enterococci. Colonies are counted using a fluorescent light and hand magnifying lens.

6.4.1 Quality Control

Enterococcus faecalis (ATCC 35550) is used as a positive control for verification of media integrity. Colonies that grow and exhibit dark blue to blue-gray pigmentation are considered as positive verification of *Enterococcus spp*.. A negative sample is performed by filtering sterile PBS and incubating along with positive control sample.

6.5 Clostridium perfringens. The membrane filtration method of Bisson and Cabelli (1979)* is used to isolate and enumerate *C. perfringens* from water samples

*Bisson, J.W., and Cabelli, V.J. 1979. Membrane filter enumeration method for *Clostridium perfringens*. Appl. Environ. Microbiol. 37 (1): 55-66.

Summary of Method

C. perfringes are analyzed by membrane filtration using mCP agar. MCP agar is prepared by dissolving 71.1 g mCP agar (acumedia 7477A) per liter deionized

water by heating to a boil while stirring with a magnetic stir bar. Dissolved media is sterilized by autoclaving for 15 minutes at 121° C, then allowed to cool to 45-50° C in a water bath. After cooling, the following ingredients are added per liter of media: 1.25 ml of a 25 mg/ml D-cycloserine solution in sterile deionized water, 250,000 units of polymyxin-B as a dissolved solution in sterile deionized water, 600 mg of indoxyl β-D-glucoside, 2 ml of a 4.5% ferric chloride solution in sterile water, and 100 mg of phenolpthalein diphosphate. Agar is then dispensed to petri dishes and allowed to solidify. Media plates are stored refrigerated for up to 2 weeks.
After water samples have been passed through membrane filters, filters are placed on mCP plates and incubated in an anaerobic chamber at 45 ± 0.2°C for 24 +/- 2 hours. The anaerobic environment is created by a commercially-available palladium catalyst pouch to which water is added or a similar device (BBL GasPak). After incubation, plates with colonies are exposed to ammonium hydroxide fumes by holding the plate inverted close to an open dish of ammonium hydroxide for 10 seconds. After exposure, colonies which turn pink to red are counted as *C. perfringens*. All other colonies are not considered C*. perfringens*. Ammonium hydroxide is discarded after each use.

6.5.1 Quality Control

Clostridium perfringens (ATCC) is used as a positive control for verification of media integrity. Colonies that grow anaerobically on MCP agar and turn from yellow to pink after exposure to ammonium hydroxide fumes are considered as a positive result for *C. perfringens* using MCP medium. A negative sample is performed by filtering sterile PBS and incubating along with positive control sample.

6.6 Coliphage*. The two-step enrichment procedure in accordance with EPA Method 1601 or the double-agar overlay method as described by Sobsey, *et al.* (1995) (EPA Method 1602).

Sobsey, M. D., D. A. Battigelli, T. R. Handzel, and K. J. Schwab. 1995. *Male-specific Coliphages as Indicators of Viral Contamination of Drinking Water.* American Water Works Research Foundation. Denver, CO.

6.6.1 Quality Control

*See section 9.13 for standard operating procedures and quality control for Methods 1601 and 1602

6.7 ***Escherichia coli*. Samples are enumerated as fecal coliforms according to method 9222D as described above. Membranes are then placed onto nutrient agar plates containing Methylumbelliferyl glucosidase (MUG) substrate and incubated for 24 hours. Colonies that fluoresce under UV light are considered to be *E. coli*.**

Summary of Method
E. coli bacteria are analyzed by membrane filtration using EC-MUG agar plates. EC

with MUG agar plates are prepared by mixing 37 g EC with MUG media and 15 g granulated agar per liter deionized water. Agar media is dissolved by boiling while stirring with a magnetic stir bar, and sterilized by autoclaving for 15 minutes at 121° C. Media is then dispensed into petri dishes, solidified agar plates are stored in the refrigerator for a maximum of two weeks.

After water samples have been passed through membrane filters, each filter is placed on an EC with MUG media plate and incubated, inverted, for 24 +/- 2 hours at 44.5 ± 0.2° C. After incubation, colonies are verified as *E. coli* by viewing the plate under a shortwave UV lamp. Colonies which fluoresce with a blue glow are counted as *E. coli*.

6.7.1 Quality Control

Escherichia coli C-3000 (ATCC 15597) is used as a positive control for verification of media integrity. Colonies that grow and fluoresce under UV light are considered as a positive result for *E. coli* using EC-MUG medium. A negative sample is performed by filtering sterile PBS and incubating along with positive control sample.

6.7.2. Overall Quality Control for Membrane Filtration Analyses

Membrane Filters – Upon receipt, each lot number of membrane filters is logged and tested for sterility by placing filter on Tryptic Soy Agar (TSA) and incubating at 35°C for 24 hours.

At least once per year, each analyst must successfully perform a blind sample and/or authentic sample that is known or has been performed by another trained analyst with statistically similar results.

6.8. **EPA Method 1623 – Detection of *Cryptosporidium* and *Giardia* in water by Filtration, Immunomagnetic Separation, and Immunofluorescent antibody.**

6.8.1. Standard Operating Procedures – SOPs for Method 1623 include internal documents P-1, P-2, and P-3.

6.8.2. Quality Control - Before analysis of any new matrix, Initial Precision and Recovery tests (spiked samples and method blanks) must be performed according to methodology and guidelines outlined in EPA Method 1623. Ongoing precision and recovery tests (Spiked samples and method blanks) are performed after every 20 samples to ensure continued proficiency in method performance.

6.9. Detection of cultivable human enteric viruses in water using filtration, concentration, and tissue cell culture - EPA Manual for Methods in Virology (600/4-84/013

6.9.1. Standard Operating Procedures – SOPs for Cultivable enteric viruses include internal documents V-1, V-2, and V-3.

6.9.2. Quality Control - Before analysis of any new matrix, Initial Precision and Recovery tests (spiked samples and method blanks) must be performed according to methodology and guidelines outlined in EPA Manual for Methods in Virology (600/4-84/013. Ongoing precision and recovery tests (Spiked samples and method blanks) are performed after every 20 samples to ensure continued proficiency in method performance.

7. Procedures for Reviewing New Work and Ascertaining Appropriateness of Facilities and Resources prior to Commencing new work

New projects and the acquisition of new clients are first reviewed by Troy Scott or Jerzy Lukasik. Project goals and outlines are discussed and appropriateness and relevance are determined. Key factors used in determining the acquisition of a new project include: 1.) Project relevance to expertise 2.) Space and time constraints 3.) Ability of laboratory personnel to complete project. 4.) Availability of equipment. 5.) Commitment of funds to project initiation.

If the above qualifications are satisfactorily met, the project is referred to Joel Caren for final approval on availability of equipment and human resources.

8. Laboratory Equipment and Calibration and/or Verification of Test Procedures Used

8.1 Laboratory equipment

The facility is equipped with a full-scale laboratory capable of performing a wide variety of analyses. The laboratory has a total of 3,600 sq. ft of research space. Equipment includes: Biological safety cabinets, laminar flow hoods, an autoclave, high speed refrigerated centrifuges, microcentrifuges, a deionized water system, Reagent grade (Milli-Q) water system, refrigerated recirculating water bath, Fecal coliform recirculating water bath, isolated tissue culture room, isolated microscopy area with epifluorescence, light, and inverted microscopes, microcentrifuges, electrophoretic power and associated gel supplies, hybridization ovens, PCR thermocyclers, fluorometers, CO_2 incubator, dry incubators, balances, pH meters, -70 C freezer, refrigerators/freezers, mixing platforms, and UV transilluminators. All are routinely certified, monitored, and/or calibrated.

8.2 Calibration and Maintenance of Laboratory equipment

8.2.1 pH meters - All pH meters are calibrated within $\pm$ 0.1 units using three point calibration (4.0, 7.0, 10.0) prior to each use and recorded in a log book. All pH calibration buffers (NIST Traceable) are aliquotted and used only once and stocks are discarded upon expiration. Electrodes are maintained according to manufacturer's instructions.

8.2.2 Balances - All balances are calibrated monthly using ASTM (NIST traceable) type weights. In addition, professional calibration of all balances occurs at least once annually.

8.2.3 Incubators – All incubators are maintained at their desired temperature ± 0.5 °C or ±0.2 °C, depending on application. Incubator temperatures are monitored using bulb thermometers immersed in glycerol, which are calibrated by a NIST traceable thermometer. Temperatures are recorded daily on log sheets. Any problems are noted on the troubleshooting log and brought to the attention of J. Lukasik, T. M. Scott, or J. Caren. Documentation must be provided as to steps taken to correct problems as they arise. The problem log is located in the QC notebook.

8.2.4 Autoclave - Each autoclave cycle is recorded in a log book that indicates the date, contents, sterilization time, temperature, and analyst's initials. A maximum temperature registering thermometer is included on each run and is recorded. Sterilization efficiency is monitored monthly using spores of *Bacillus stearothermophilus* as a control.

8.2.5 Sterilization procedures - All items are sterilized in the autoclave at 121°C for a minimum of 15 minutes. Biohazardous wastes are sterilized for a minimum of 30 minutes.
8.2.6 Refrigerators - All refrigerators/freezers are monitored to maintain a temperature of1-8 °C by a bulb thermometer immersed in glycerol.

8.2.7 Conductivity meters - All conductivity meters are capable of measuring conductivity within 1 uOhm per centimeter. Lab and reagent-grade water are routinely tested for conductivity. Conductivity meters are calibrated monthly using a certified, traceable standard.

8.3 Procedures for Achieving Traceability of Measurements

All measurements by analytical equipment are recorded and dated by each user after use. Log sheets are filed for reference for up to 3 years.

8.4 Quality assurance of accuracy and precision of data

Quality assurance (Internal standards, blind samples, duplicate samples) measures are listed with individual SOPs within the QA document.

9. Laboratory setup and procedure

9.1 Laboratory setup and environment

9.1.1 Bench space - All laboratory areas have sufficient bench space for reagent and supply storage and operation of equipment. Excess space is available for performing laboratory work.

9.1.2 Lighting - sufficient overhead fluorescent lighting is present in each room. Emergency lighting that has its own power supply is also present in each room.

9.1.3 Air system - The laboratory temperature is maintained by a 2.5 ton air handler;

temperature is controlled from a central location and maintained at a maximum of 25 ^{o}C. Hepafilter room air purifiers are used to reduce the number of airborne particulates in the air and to improve the laboratory air quality.

9.1.4 Waste disposal - Routine materials are autoclaved and placed in trash and sent to landfill; infectious wastes and potential pathogens are collected in specialized containers and marked to be sent for incineration.

9.1.5 Safety considerations - General safety procedures are followed: Lab coats and gloves are worn; infectious agents are handled in a biological safety cabinet. Chemical waste is stored in designated containers and appropriate safety cabinets are used for storage of chemicals.

9.1.6 Chemicals - All chemicals and reagents are stored in clearly labeled bottles and labeled with date when opened and are discarded according to manufacturer's instructions. Precautions and reactivity are indicated on storage containers. Chemical waste is stored in designated labeled containers and sent for appropriate disposal. Safety cabinets are used for storage of chemicals. Materials Safety and Data Sheets (MSDS) are filed and are available for reference by lab personnel.

9.2 Field Sampling (EPA Manual for Methods in Virology (600/4-84/013) and EPA Method 1623; SOPs V-1, P-1)

The procedures for collection and processing of well water, surface water, and wastewater for virus and parasite analysis according to the EPA Manual for Methods in Virology (600/4-84/013) and EPA Method 1623, respectively, are as follows:

1. Samples are collected from a tap or using a gasoline driven pump connected to a water source or large plastic 100 gallon container. If necessary, a separate container remains at each designated site or for a series of related sites. All containers, sampling tubing, pumps, and filter housings are disinfected prior to use by the passage of a 10% bleach solution with a 1 minute contact time. The chlorine residual is then neutralized by the passage of a 5% solution of sodium thiosulfate.

2. Field data sheets are used to collect information on the sampling site. Information includes identification of the site, volumes collected, time of collection, technician's name, and any deviations from standard procedures.

3. If necessary, chlorinated effluent samples are collected in the 100-gallon tank and are dechlorinated by the addition of 4mL of a 10% sodium thiosulfate solution. This is sufficient to neutralize up to 10 mg/L of free chlorine in 400L (approximately 100 gals.).
4. For filtration of virus samples, 1MDS filters (Cuno, Inc) are aseptically placed into a filter holder connected to the water pump tap. Gelman Envirocheck HV filters are used for parasite analyses. A flow meter is attached to the outlet side of the filter to record the volume of water passed through the filter. The sample is pumped through the filter at a

rate of 1.5-5 gallons per minute.

5. After the desired volume has been passed through the filter, the filter is removed, packaged in a sterile whirl-pak, labeled, placed on ice for delivery to the laboratory, and processed within 24 hours.

9.3 Elution, Concentration, and Detection of Enteroviruses and Protozoan Parasites

Virus analysis – SOP V-2

1. Filters are eluted using 1L of 1.5% beef extract + 0.05M glycine (pH 9.5) by the procedure and apparatus described in the U.S. EPA ICR Microbiology Manual.

2. Filter eluants are concentrated by organic flocculation as described by the manual. Sample concentrates are then transferred to several plastic tubes, supplemented with antibiotic/antimycotic, and frozen until assayed by methodology described below.

3. Inoculation onto tissue cell culture flasks is performed in accordance with the procedure described tin the EPA ICR Microbial Laboratory Manual with some modification as described below.

Parasite analysis and Quality Control – SOP P-2

Gelman Filters are processed according to EPA Method 1623 with immunomagnetic separation and concentration (SOP P-2). The resulting concentrated pellets are examined by microscopic analysis. Results are reported as number of (oo)cysts per 100L.

9.3.1 Quality Control

All virus and parasite filters are processed using aseptic technique, using decontaminated hoses and filter housings. Envirochek filters (for use with method 1623) are self-contained and can be eluted *in situ*. In addition, negative controls for virus processing are accomplished by processing a sterile 1MDS filter as described above. Positive virus controls are accomplished by a matrix spike (wastewater, surface water, finished water) with approximately 200 PFU of attenuated poliovirus Lsc1. Positive control filters are processed in a separate room with separate equipment. Recovery efficiencies of 50% or greater are considered satisfactory. For parasites, negative controls are routinely processed with every 20th sample beginning with the IMS step of the procedure. Positive controls are accomplished by matrix spikes according to procedures outlined in EPA Method 1623 using cysts and oocysts enumerated by flow cytometry (SOP P-3).

9.4 Bacteriological assays

9.4.1 Grab Sampling – Water samples for bacteriological assays are collected by the grab sample method as in Standard Methods for the Examination of Water and Wastewater

(9060A). Polyethylene bottles are pre-sterilized by autoclaving and closed with a screw-cap lid. Sampling technicians are to wear latex gloves and change gloves between each sample collection. All specimens collected for projects are labeled properly in the field with sampling site, date and time of collection and initials of person collecting. A field log sheet shall accompany all samples with all needed information documented on the form. The time specimens are received back in the laboratory is documented on the field log sheet along with the initials of person receiving specimens.

9.4.2 Membrane Filtration equipment - All membrane filtration manifolds are constructed of stainless steel or custom made from PVC and are certified by the manufacturer for total coliform analysis or verified for proficiency by authorized laboratory personnel prior to use.

9.4.3 Membrane filters - All filters are cellulose ester, white, gridmarked, 47mm in diameter, 0.45 micron pore size, and are purchased pre-sterilized. Lot numbers of all membrane filters are recorded and filed.
9.4.4 Culture dishes - Presterilized plastic culture dishes are used for all membrane filters and for routine bacterial analyses.

9.4.5 Culture tubes - All culture tubes are made of polyethylene or borosilicate glass and are closed with plastic caps

9.4.6 Sample containers - Sample containers are wide mouth plastic bottles with airtight caps or whirl-pak sterile plastic bags.

9.4.7 Laboratory bacterial control strains - Positive controls for the various assays are the following: Fecal coliform, total coliform, and *E. coli* – *E. coli* ATCC #15597
Enterococci – *Enterococcus faecalis* ATCC #35550
C. perfringens – *C. perfringens* ATCC #3624

Stocks are obtained from the American Type Culture Collection and maintained by initially re-hydrating the freeze-dried culture and propagating according to ATCC instructions for that organism. Once a high-concentration broth culture of the organism has been grown, aliquots of the suspension are mixed at a 1:1 ratio with Dimethylsulfoxide (DMSO) in 1 mL cryovials and preserved frozen at –70° C.

9.5 <u>Viable enterovirus assays – SOP V-3</u>

Cell culture - all tissue cell cultures are passed every 4-6 days routinely to maintain line. Sample Inoculation onto tissue culture cells - Each sample is inoculated onto low passage number (<80) BGM, RD, and MA104 cells according to procedures described in the EPA Manual for Methods in Virology (EPA 600/4-84/013). All inoculated flasks are maintained for at least 14 days followed by passage onto fresh monlayers and an additional 14 day incubation.

9.5.1 Quality Control

Confirmation/verification of positive results - all inoculated cell cultures with or without visible cytopathic effects are frozen at -70 C, thawed, filtered through a 0.22 micron porosity filter, and used to reinoculate fresh cell cultures.

Positive controls – In addition to matrix spikes, natural samples containing high levels of enteric viruses (raw domestic sewage, primary sludge) are sampled during the treated source sampling and assayed as positive controls. All positive control samples are collected and maintained using separate pumps, housings, hoses, and elution apparatuses. In addition, poliovirus 1 (strain Lsc) is used as a positive control in all cell cultures.

Negative control – In addition to filter blanks, cell cultures are also routinely maintained during experiments as negative controls. These cells are inoculated with sterile deionized water or phosphate buffered saline (PBS) and maintained along with inoculated samples. CPE observed in negative controls indicates contamination or loss of integrity of cell line.

9.6 Microscopic examination of *Cryptosporidium and Giardia and Cryptosporidium* cell culture viability assay

Microscopic examination of *Cryptosporidium* and *Giardia* parasites after staining with FITC and DAPI is performed using an epifluorescence microscope. As an additional verification, organisms are also verified by Nomarski Differential Interference Contrast (DIC) microscopy.

Viability of *Cryptosporidium* oocysts is evaluated using the method described by Slifko et al. (1997). Briefly, Human ileocecal adenocarcinoma (HCT-8) cells are plated into 8 well tissue culture slides and grown to confluence. Samples are washed in 0.525% reagent grade sodium hypochlorite at 4 degrees C for 8 minutes for purposes of sterilization and to enhance excystation of the oocysts. Samples are then added to the well slides and incubated at 37°C and 5% CO_2 for 48 hours. After incubation, the tissue cells are fixed with 100% methanol and viable parasites are detected using a labeled antibody technique described previously (Slifko et al. 1999). Slides are then viewed by epifluorescence and Differential Interference Contrast (DIC) microscopy for multiple life stages of the parasite.

9.6.1 Quality Control

Positive Controls – positive controls using infectious *C. parvum* oocysts are performed prior to microscopic examination of filter concentrates and during each viability experiment. For viability testing, this QA measure ensures that the HCT-8 cells have retained the ability to become infected, as well as verifying the ability of the antibody to identify life stages of the parasite. For direct microscopic examination of *Giardia* cysts and *Cryptosporidium* oocysts, this QA measure verifies integrity of the monoclonal antibody against these structures.

Negative control – wells containing monolayers of HCT-8 cells are inoculated with sterile phosphate buffered saline (PBS), incubated, fixed, stained, and examined microscopically to ensure the absence of infectious foci or the appearance of infectious foci in wells that do not contain *Cryptosporidium* oocysts. Method blanks are included every 20 samples processed according to requirements outlined in EPA Method 1623.

9.7 Host Associated Molecular Markers (PCR and Quantitative PCR based assays)

Filtration of samples - A specified volume of water from each sample (*Enterococcus* or *Bacteroidetes)* is filtered through a 0.45 micron filter to collect bacterial cells for molecular analysis. In case of a clogged filter, an additional filter is utilized until an appropriate volume of water is filtered. Each filter is then processed according to methodology outlined below.

Preparation of *Enterococcus* template DNA for PCR and qPCR reactions - PCR and qPCR reactions are performed on composite DNA samples extracted from membrane filters. Filters containing enterococci colonies are lifted, suspended in Azide dextrose broth (Difco), vortexed vigorously, and incubated for 3 hours at 41 ^{o}C to wash bacteria from the filters and partially enrich the culture. DNA extraction is performed on the resulting culture of bacteria using a Qiagen Stool DNA extraction kit according to manufacturer's instructions (Qiagen, Inc.).

Preparation of *Bacteroidetes* template DNA for PCR and qPCR reactions - PCR and qPCR reactions are performed on composite DNA samples extracted from membrane filters. Water samples are filtered and filters are lifted, suspended in Qiagen Stool Lysis Buffer and vortexed vigorously. The resulting lysate is processed for DNA extraction according to manufacturer's instructions (Qiagen stool DNA extraction kit).

PCR primers and reaction conditions for Human *Enterococcus* marker - Primers specific for the *esp* gene in *E. faecium* were developed by Scott et al. (2005). The forward primer, which is specific for the *E. faecium esp* gene is: (5'-TAT GAA AGC AAC AGC ACA AGT T-3'). A conserved reverse primer (5'-ACG TCG AAA GTT CGA TTT CC-3'), developed previously by Hammerum and Jensen, was used for all reactions. PCR reactions were performed in a 50 uL reaction mixture containing 1X PCR buffer, 1.5 mM $MgCl_2$, 200 uM of each of the four deoxyribonucleotides, 0.3 uM of each primer, 2.5 U of HotStarTaq DNA polymerase (Qiagen), and 5 ul of template DNA. Amplification was performed with an initial step at 95 ^{o}C for 15 minutes (to activate Taq polymerase), followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. PCR products were separated on a 1.5% agarose gel stained with GelStar nucleic acid stain (BioWhittaker) and viewed under UV light. The PCR product is 680 base pairs in length.

qPCR primers and probes and reaction conditions for Total *Enterococcus* marker and Human *Enterococcus* marker – qPCR primers specific for the esp gene in *E. faecium* were developed based on Scott et al. (2005). The forward primer, which is specific for the *E. faecium* gene is: 5'- TATGAAAGCAACAGCACAAGTT-3' and is identical to the forward

primer for conventional PCR. The reverse primer is: 5'- TATGAAAGCAACAGCACAAGTT-3' and the sequence for the "Taqman" probe is 5'-CCATTTGGTGAAGATTTCATCTTTGATTC-3'. The probe is labeled with FAM at the 5' end and TAMRA at the 3' end.

A qPCR assay for the total *Enterococcus* marker was designed based on the target sequence described by Cheng et al. (Journal of Clinical Chemistry, 35, 1248-1250, 1997). The marker sequence was lodged with GenBank under accession number L78127. The forward primer is: 5'-GTTGGTGCAGCTGTGCCA-3'; the reverse primer is: 5'-CGAACGCGACCGTCATG-3'; the sequence for the "Taqman" probe is 5'-CCAAATCGATCCGCATCCATGATCA-3'. The probe is labeled with FAM at the 5' end and TAMRA at the 3' end.

qPCR reactions for both the human *Enterococcus* marker and total *Enterococcus* marker were performed in 25ul reaction mixtures containing 1X Taqman Universal Master Mix (Applied Biosystems) with 900nM of both forward and reverse primers and 250nM probe and 2ul of template DNA. Amplification and analysis was undertaken in an Applied Biosystems 7700 with the following amplification conditions: 50°C for 10 minutes and 95°C for 15 seconds followed by 40 cycles of 95°C for 15 seconds and 57°C for 1 minute. Gene marker copy numbers were calculated by reference to standard curves generated using synthetic target amplicons.

PCR primers and reaction conditions for Human and Ruminant *Bacteroidetes* marker - Primers specific for *Bacteroidetes* derived from human and ruminant sources were developed by Bernhard and Field (2000). PCR reactions were performed according to methodology outlined by Bernhard and Field (2000).

Bernhard, A.E., and K.G. Field. 2000. A PCR assay to discriminate human and ruminant feces based on host differences in *Bacteroides-Prevotella* 16S ribosomal DNA. Appl. Environ. Microbiol. 66: 4571-4574.

Scott, T.M., T.M. Jenkins, J. Lukasik, and J.B. Rose. 2005. Potential use of a host-associated molecular marker in *Enterococcus faecium* as an index of human fecal pollution. *Environ. Sci. Tech*. 39: 283-287.

qPCR primers and reactions for the Human *Bacteroidetes* marker- qPCR primers specific for *Bacteroidetes* derived from human sources were developed by Seurinck et al (2004). qPCR reactions and gene copy number calculations were performed according to the methodology of Seurinck et al (2005).

Seurinck, S., T. Defoirdt, W. Verstraete and S.D. Siciliano. 2005. Detection and quantification of ythe human-specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. Environmental Microbiology 7(2), 249-259.

9.7.1 Quality Control

All molecular biology is performed in an isolation room separated from live bacterial, viral, and tissue cell cultures.

Experiments are performed using separate pipettors with aerosol resistant tips, latex gloves are used at all times, and equipment and bench space is routinely wiped down with DNAse and RNAse inhibitors.

Negative and positive controls are used in all PCR reactions. All DNA extractions are performed using kits that remove PCR inhibitors. When applicable, negative samples are spiked with positive control DNA to assess the presence of PCR inhibitors.

9.8 Procedures for Testing Turbid Waters for Fecal Contamination

9.8.1 Estimation of fecal coliform bacteria by MPN method

Fermentation tubes containing A-1 medium (Difco) and Durham tubes are arranged in three rows of five tubes each, with the first row of five containing double-strength medium (2X). Each row of tubes is inoculated with a dilution of water (usually 10mL, 1.0mL, and 0.1mL). Replicate samples are also inoculated.

Tubes are then incubated in a water bath at 35 $\pm$ 0.5 ^{o}C for 3 hours. Tubes are then transferred to a 44.5 $\pm$ 0.2 ^{o}C water bath and incubated for an additional 21 $\pm$ 2 hours.

Positive tubes are identified by the presence of growth and gas bubble in the Durham tube.

9.8.2 Internal Quality Control

Tubes are inoculated with sterile deionized water and incubated as above. In addition, a known dilution of *E. coli* is inoculated into tubes and MPN is verified. For negative controls, a known volume of *Enterobacter aerogenes* is inoculated into the tubes and the tubes are observed for the absence of growth.

9.8.3 Calculation of MPN

MPN can be calculated either by referencing standard MPN tables or by using the following formula:

$$\frac{\text{\# of positive tubes X 100}}{(\text{mL negative X total mL assayed})^{0.5}}$$

Note: Isolation of *E. coli* from these samples is achieved by streaking from a positive tube and following protocol outlined for isolation of *Escherichia coli* under ribotyping procedure.

9.9 Procedures for Testing Potable Waters for Fecal Contamination

9.9.1 Estimation of Fecal Coliform Bacteria by Membrane Filtration

When the density of fecal coliform bacteria is unknown, several decimal volumes are filtered.

Filter funnels are washed and triple rinsed, with a final rinse in deionized water. Funnels are then wrapped in aluminum foil and sterilized by autoclaving at 121° C for 15 minutes. The filter setup is carefully placed upon a collection vessel connected to a vacuum pump. With the sterile filter in place, the funnel is filled with a 20-30mL portion of sterile phosphate-buffered saline (PBS) or sterile 0.1% peptone. A dilution of sample to be tested is then added to the funnel and is filtered by vacuum. After filtration, the funnel is rinsed three times with sterile water or PBS. Upon completion of the final rinse, the vacuum is disengaged, the funnel is removed and the membrane is carefully removed with flame-sterilized forceps and placed on an appropriate medium (usually m-FC medium, DIFCO).

9.9.2 Internal Quality Control

Between every sample, sterile water is filtered and placed on an appropriate medium. At the end of the filtration of samples, a known dilution of *E. coli* is filtered and verified.

9.9.3 Incubation

Plates are placed into waterproof plastic bags or sealed petri dishes and submerged in a water bath for incubation at 44.5 ± 0.2 °C for 24 ± 2 hours. Fecal coliform colonies appear as various shades of blue on m-FC agar and can be counted directly for estimation of bacterial density. Blue colonies are then verified as *E. coli* by the methods described for isolation of *Escherichia coli* under ribotyping procedure.

9.10 Water Sample Processing for Isolation of *Escherichia coli*

All samples are processed in a separate location from the sample storage room. All samples are processed individually and sample identification numbers are recorded when removed from the storage facility and again at the processing station. Unused samples are stored in a separate refrigerator and the sample identification number is again verified and recorded.

9.10.1 Drinking water

Drinking water samples or samples exhibiting low turbidity are analyzed using the membrane filtration technique and incubation at 44.5°C (SM9222D). Dark blue colonies are enumerated (for analysis of fecal coliforms), inoculated into Tryptic Soy Broth (TSB) containing MUG substrate (Sigma), and verified using the IMVIC series of tests.

9.10.2 Wastewater and Surface Water

Wastewater or highly turbid surface waters are enriched at 44.5°C in A1 media (Difco) and enumerated using the Most Probable (MPN) Technique (SM9221E). Tubes exhibiting positive growth and gas production are then streaked onto MacConkey plates and processed as described below for identification of *Escherichia coli*.

9.11 Fecal Sample Processing for Isolation of *Escherichia coli*

Fecal samples are streaked directly onto MacConkey agar plates (Difco) or suspended into phosphate buffered saline (PBS, pH 7) prior to streaking. Plates are incubated at 44.5°C and lactose-positive colonies are picked and subcultured into Tryptic Soy Broth (TSB, Difco) containing MUG substrate (Sigma). MUG-positive isolates are presumed to be *E. coli* and are verified using the IMViC series of tests (Indole, Methyl Red, Voges-Proskauer, Citrate). Isolates exhibiting ++-- IMViC profiles are confirmed as *E. coli*. Alternatively, samples are streaked onto EC agar plates containing MUG substrate and incubated at 44.5°C. Colonies exhibiting fluorescence under UV light are then verified as *E. coli* using the IMViC series of tests.

9.12 Selection of *E. coli* Reference Strains

Several well-characterized human and non-human derived *Escherichia coli* from our extensive collection were used in the establishment of an original database for isolate classification. These reference strains are valuable for verifying sources of *E. coli* as being either human or animal-derived and are included as internal controls to measure genotypic variation over time. All isolates are maintained in liquid nitrogen.

9.13 Bacteriophage assay

Bacteriophage assays are performed according to EPA Methods 1601 and 1602.

9.13.1 Method 1601 – The two step enrichment procedure described in this method determines the presence or absence of male specific and somatic coliphages in ground water and other waters. In addition to being used for presence absence, the method may be used as a quantitative assay provided an appropriate MPN procedure is used. However, the method has not been validated this way. Briefly, 100mL or 1L water samples are supplemented with $MgCl_2$, log phase host bacteria (*E. coli* CN-13 for somatic bacteriophage, *E. coli* F_{amp} for male-specific bacteriophage) and Tryptic Soy Broth in the initial enrichment step for each type of coliphage. After an overnight incubation , samples are spotted onto a lawn of host bacteria, incubated, and examined for circular lysis zones, which indicate the presence of coliphage.

9.13.2 Method 1602 – The single agar overlay procedure is designed to detect and enumerate male-specific and somatic coliphages is ground water and other waters. Briefly, a 100mL water sample is assayed by adding $MgCl_2$, log phase host bacteria (same as above), and 100mL of double strength molten tryptic soy agar (TSA) to the sample. The sample is thoroughly mixed and the total volume is poured into sterile petri plates. After overnight incubation, circular lysis zones are counted and summed for all plates. The quantity of coliphage is expressed as plaque forming units (PFU)/100mL. A variation of this technique is the double agar overlay procedure (Snusted and Dean) in which samples are diluted and added to tubes containing host bacteria and single strength molten TSA. This method is used when coliphage titers are high and small diluted volumes need to be

assayed.

9.13.3 Quality Control – In addition to above host bacteria, *E. coli* C-3000 (ATCC 15597) is used as a host that is susceptible to both somatic and male-specific coliphage. Use of this host allows for an internal measure of both types of bacteriophage in order to cross-reference results. This host organism is routinely used for all bacteriophage analyses.

The integrity of each host and its ability to be infected by somatic and F-specific coliphage is verified by using positive controls. PhiX-174 is used as the somatic coliphage positive control. Bacteriophage MS-2 is used as the male-specific positive control. Plaque formation on a host lawn is regarded as verification of host integrity.

10. Ribotyping Procedure

10.1 DNA Extraction

Verified *E. coli* isolates are grown overnight in Tryptic Soy Broth (TSB) and DNA is extracted using the Easy DNA kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions.

10.2 Determination of DNA Concentration

DNA concentration is determined using a TKO 100 fluorometer according to manufacturer's instructions.

10.3 Restriction Enzyme Digestion

Approximately 1 microgram of DNA is digested with *HindIII* restriction enzyme (Promega) according to manufacturer's instructions. Digested DNA is separated on a 1.0% agarose gel at 30 V for 16 hours in TBE (Tris-Borate-EDTA) buffer, stained with ethidium bromide, and viewed under UV light.

10.4 Southern Blot Analysis

After electrophoresis of restriction-digested DNA, agarose gels are denatured in 0.5M NaOH/1.5 M NaCl for 35 minutes and neutralized in 0.5 M Tris-HCl (pH 7.2)/1.5 M NaCl (0.0001 M) disodium EDTA for 45 minutes. DNA is blotted from gels onto nylon membranes (BioRad) using a vacuum blotting system (VacuGene XL) and fixed with shortwave UV light for 5 minutes.

10.5 Probe Preparation

E. coli 16S and 23S rRNA is reverse transcribed into cDNA with avian reverse transcriptase and labeled with digoxigenin–dUTP according to the manufacturers instructions (Roche Molecular Diagnostics, Mannheim, Germany).

10.6 Hybridization and Detection

Membranes are prehybridized at 42 ^{o}C for 2 h and then hybridized with the digoxigenin-labeled probe at 65°C for 16 hours. After hybridization, membranes are washed twice for 5 min. each time with 2X SSC (0.3M NaCl, 30mM sodium citrate)/ 0.1% SDS at room temperature for 15 minutes and twice for 15 min. each time at 65°C with 0.5X SSC-0.1% SDS. Membranes were then reacted with alkaline phosphatase conjugated anti-DIG antibody and visualized by using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate for colorimetric detection according to the manufacturer's instructions (Roche Molecular Diagnostics, Mannheim, Germany).

10.7 Statistical Analysis

RT banding profiles are read by hand and DNA fragments are translated into binary code. Binary codes are examined using Bionumerics statistical discrimination methodology. Results of the discrimination model are summarized by use of the average rate of correct classification (ARCC) and the percentage of correctly and misclassified isolates from the classification table.

10.8 Internal QC Standards

E. coli ATCC 9637 is used as an internal standard during all RT analyses. The banding pattern of this organism is known and ribotypes exhibiting alternative profiles are discarded. Additional, well-characterized human and nonhuman *E. coli* from our collection and reference database are also routinely ribotyped to verify consistency of discriminating classification. Note: Classification percentages of isolates cannot be held constant, as they are a function of the diversity of known-source RT profiles in the reference database.

10.9 Input of RT data into Bionumerics program

RT bands are read by hand and transcribed into paper copy as a series of 1's and 0's in one of 32 boxes designating possible band positions. The hard copy is then typed into a computer file (notepad) and includes sample number along with the binary codes. The binary code is then verified to be identical to the hard copy form for each individual sample. This information is then opened in the Bionumerics program using a password and is cross-referenced in the database. The information at no time enters the reference database and the information cannot compromise the database. The reference database is password-protected and maintained separately by a statistician. He first analyzes new additions to the database, and only well-characterized strains (not experimental) are used for increasing its size.

11. Procedure for Handling Collected and Submitted Samples

11.1 Submitted Samples

11.1.1 Transport of samples - All samples are received cold or on ice and temperature is verified upon receipt by measuring temperature of ice or water in the shipping container. Once received, are immediately labeled, recorded, analyzed, then refrigerated.

11.1.2 Holding times - for all bacteriological and virus samples are a maximum of 24 hours. Parasite samples have a 36 hour hold time and are usually processed within 24 hours.

11.1.3 Sample storage - Beef extract viral eluates are stored at -70 C until inoculation. Water samples are maintained at 4°C and analyzed upon receipt.

11.1.4 Record keeping - Laboratory worksheets and notebooks are maintained to record sample information. Sample information is recorded and contains the following information:

1. Name of sample site
2. Sample identification
3. Sample type
4. Date and time of collection
5. Analyses required
6. Chlorine residual (if any)
7. Name of sampler and organization
8. Transportation condition (temperature, protection from sunlight, etc.)

11.2 Chain of Custody Forms - When applicable, chain of custody forms are used when samples are transferred between parties. These forms follow state-applicable guidelines and are filed upon receipt.

12. Corrective action contingencies

Unacceptable results - if unacceptable results are obtained with PE or QC samples, tests with additional positive and negative controls are conducted after calibration of all equipment used in the procedure to determine the source of the problem. The laboratory directors (J. Lukasik, T.M. Scott) or Sr. Research Scientist (J. Caren) will take corrective action if necessary.

Departure from documented procedures or standard specifications - If a prescribed methodology is deemed to be inaccurate or unreliable for a particular sample, alternative methodologies will be independently pursued by either Dr. Lukasik or Dr. Scott. If results from explorative research are consistent, standard procedures may be modified in the existing case. Deviation from standard procedure must be approved by Dr. Lukasik or Dr. Scott.

13. Procedures for Dealing with Complaints

Complaints are received, documented, and referred to Dr. Lukasik or Dr. Scott. Should errors be found, they are corrected and clients are notified. If no errors are found, clients

are contacted to investigate the source of the problem or complaint. Corrective actions are taken to assure the accuracy and reproducibility of the results, and the satisfaction of the customer with the testing protocol.

14. Procedures for data reduction, verification, validation, and reporting of results

Data reduction - All statistical analyses are performed using analytical computer software. Results are compiled into reports and are stored as a hard copy and in a computer database, and backed up by external electronic storage devices.

Accuracy of transcriptions - Sample collection sheets and laboratory data sheets are compared and verified before report preparation and are saved and available for confirmation of results.

Data Validation - The laboratory directors will monitor compliance with internal audits and previously set EPA ICR QC requirements
Reporting - copies of all data, reports, and monitoring forms as well as final reports are supplied to the primary investigator and filed for further use.

15. Procedures for establishing that personnel are adequately trained and procedures used for training purposes

Training of personnel - All personnel are trained on use of all equipment prior to beginning work by Jerzy Lukasik, Troy Scott, or Joel Caren.

Training on new equipment or procedures - All personnel are trained on new equipment or procedures as is necessary. Initially and routinely, all personnel are tested for their knowledge, and are trained and familiarized with standard research and safety practices. Employees are encouraged to research new protocols and novel procedures to enhance lab productivity and minimize cost.

Training on ethical and legal responsibilities - All personnel are trained on proper laboratory procedures with regards to ethical and legal rights and responsibilities.

All lab personnel are provided with access to the Laboratory QA/QC plan. All personnel are required to read and sign the document before beginning work. Drs. Lukasik and Scott have individual copies of the plan for reference. Revisions to the plan are documented with date and are recorded directly on the document.

16. Procedures for protecting confidentiality and proprietary rights

Confidentiality agreements - Service work performed in exchange for pay is maintained confidential. The clients identity and nature of the work will be protected and not be disclosed to any out side parties. Analysis results will only be disclosed to the party submitting the sample or requesting the analysis. Typically, When parties of two or more

are involved in a project in which integrity must be strictly maintained, confidentiality agreement are signed and witnessed by all parties.

17. Record Keeping and reporting of results

16.1 Recordkeeping - Records are maintained in bound notebooks and on IBM-compatible Zip disks or CDR disks. Electronic back up copies of all files are maintained in a different location and on a canalized computer that is connected to emergency power back system. All records are stored for a minimum of 5 years. Records include raw data, calculations, and quality control data.

16.2 Reporting of Results - All clients are notified promptly of a positive result (total coliform, fecal coliform, *E. coli*, enterovirus, protozoan parasite) so that appropriate follow-up actions can be conducted. Results are reported as direct quantitative counts or most probable number of organisms per sample. Reports include methodology used, limits of detection, positive and negative controls used, overall results, and interpretation of final results.

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Human Bacteroidetes ID™

Detection of the Fecal *Bacteroidetes* Human Gene Biomarker for Human Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: XYZ Municipal Beach
Submitter #'s: 575, 576, 577 and 578
Source Molecular #'s: SM 0525, SM 0526, SM 0527 and SM 0528
Samples Received: May 25, 2004
Date Reported: June 02, 2004

SAMPLE

SM #	Client #	DNA Analytical Results
SM 0525	575	Human Gene Biomarker Detected
SM 0526	576	Negative
SM 0527	577	Human Gene Biomarker Detected
SM 0528	578	Negative

Laboratory Comments

The submitted water samples were filtered for fecal *Bacteroidetes.* The filters were then eluted and centrifuged for DNA analysis. Fecal *Bacteroidetes* are found in abundant amounts in feces of warm-blooded animals. They are considered a good indicator of recent fecal pollution because they are strict anaerobes (i.e. they do not survive long outside the host organism).

All reagents, chemicals and apparatuses were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminates, including PCR inhibitors.

Samples 576 (Our Ref: SM 0526) and 578 (Our Ref: SM 0528) tested negative for the fecal *Bacteroidetes* human gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have human contamination. In order to strengthen the result, a negative sample should be analyzed further for human fecal contamination with other DNA analytical tests such as the Human Enterococcus ID™ and Human Fecal Virus ID™ services.

Samples 575 (Our Ref: SM 0525) and 577 (Our Ref: SM 0527) tested positive for the fecal *Bacteroidetes* human gene biomarker suggesting that human fecal contamination is present in these water samples. The client is nonetheless encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the results.

DNA Analytical Method Explanation

Water samples (100 ml each) were filtered through 0.45 micron membrane filters. The filters were placed in separate 50-ml disposable centrifuge tubes containing 5 ml of lysis buffer (20 mM EDTA, 400 mM NaCl, 750 mM sucrose, 50 mM Tris; pH 9).[2]

DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturers instructions. Five micro-liter aliquots of purified DNA extraction were used directly as template for subsequent PCR reactions. Amplification of PCR primers were carried out using HotStarTaq polymerase (Qiagen, Inc.) and master mix, which contained a final concentration of 1.5 mM MgCl2, 150 mM dNTP, and 0.3 mM of each primer.

An Eppendorf Gradient Thermocycler was used with the following cycling parameters: 25 cycles of 94°C for 30 s, appropriate annealing temperature for 30 s, and 72°C for 1 min followed by a final 6-min extension at 72°C. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Biowhittaker, Inc.) and visualized under UV light.

DNA Analytical Theory Explanation

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately found in humans. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found to be specific to humans.[2,3] As such, these bacterial strains can be used as indicators of human fecal contamination.

One of the advantages of the Human Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection by gel electrophoresis.

The gel electrophoresis apparatus uses an electrical field to distinguish different DNA fragments according to their molecular weights. Lighter DNA fragments will move farther along the gel than their heavier counterparts. At the end of the procedure different bands of accumulated DNA fragments will aggregate at different parts of the gel. It is this accumulation of DNA fragments that creates a band on the gel. Researchers use these bands to distinguish certain genomes such as the human gene biomarker from the *Bacteroides* and *Prevotella* genus.

These banding patterns confirm or negate the presence of the fecal *Bacteroidetes* human gene biomarker. As such, the banding patterns provide a reliable indicator of human fecal contamination. To strengthen the validity of the results, the Human Bateroidetes ID™ service should be combined with other DNA analytical services such as the Human Enterococcus ID™ and Human Fecal Virus ID™ services.

[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

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[4] Kreader, C.A. (1995). **Design and evaluation of Bacteroides DNA probes for the specific detection of human fecal pollution.** Applied and Environmental Microbiology, 61: 1,171-1,179.

[5] Kreader, C.A. (1998). **Persistence of PCR-detectable Bacteroides distasonis from human feces in river water.** Applied and Environmental Microbiology, 64: 4,103-4,105.

[6] Dick, Linda K., Field, Katharine G.**Rapid Estimation of Numbers of Fecal Bacteroidetes by Use of a Quantitative PCR Assay for 16S rRNA Genes.** Appl. Environ. Microbiol. 2004 70: 5695-5697.

Limitation of Damages – Repayment of Service Price

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Human Enterococcus ID™

Detection of the *Enterococcus faecium* esp Human Gene Biomarker for Human Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: ABC Beach Park
Submitter #'s: 775, 776, 777 and 778
Source Molecular #'s: SM 0125, SM 0126, SM 0127 and SM 0128
Samples Received: May 19, 2003
Date Reported: May 23, 2003

SAMPLE

SM #	Client #	Enterococci (CFU/100mL)[7]	Enterococci Analyzed	DNA Analytical Results
SM 0125	775	45	180	Human Gene Biomarker Detected
SM 0126	776	150	600	Negative
SM 0127	777	255	1020	Human Gene Biomarker Detected
SM 0128	778	15	60	Negative

Laboratory Comments

The submitted water samples were filtered for *Enterococcus spp.* and the *Enterococci* were enumerated on petri plates. Afterwards, the *Enterococci* were eluted and centrifuged directly from the filter for DNA analysis.

All reagents, chemicals and apparatuses were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminates, including PCR inhibitors.

Samples 776 (Our Ref: SM 0126) and 778 (Our Ref: SM 0128) tested negative for the *Enterococcus faecium* human gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have human contamination, particularly when the total *Enterococci* is less than 100 total viable cells (see forth column and DNA Analytical Theory Explanation section). In order to strengthen the result, a negative sample should be analyzed further for human fecal contamination with other DNA analytical tests such as the Human Bacteroidetes ID™ and Human Fecal Virus ID™ services.

Samples 775 (Our Ref: SM 0125) and 777 (Our Ref: SM 0127) tested positive for the *Enterococcus faecium* human gene biomarker suggesting that human fecal contamination is present in these water samples. The client is nonetheless encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the results.

DNA Analytical Method Explanation

100 ml of water was filtered through 0.45micron membrane filters. Filters were placed on mEnterococcus media supplemented with indoxyl substrate and plates were incubated for 24 hours at 41°C according to the protocol outlined in EPA Method 1600.[7] Colonies exhibiting a blue halo were enumerated as *Enterococci*.

DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturers instructions. Five micro-liter aliquots of purified DNA extraction were used directly as template for subsequent PCR reactions. Amplification of PCR primers were carried out using HotStarTaq polymerase (Qiagen, Inc.) and master mix, which contained a final concentration of 1.5 mM MgCl2, 150 mM dNTP, and 0.3 mM of each primer.

An Eppendorf Gradient Thermocycler was used with the following cycling parameters: 95°C for 15 minutes (to lyse cells and activate polymerase), followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute and a final extension at 72°C for 5 minutes. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Biowhittaker, Inc.) and visualized under UV light.

DNA Analytical Theory Explanation

Enterococci are a subgroup of Fecal *Streptococci* and are characterized by their ability to grow in 6.5% sodium chloride, at low and elevated temperatures (10°C and 45°C), and at elevated pH (9.5). These microorganisms have been used as indicators of fecal pollution for many years and have been especially valuable in the marine environment and recreational waters as indicators of potential health risks and swimming-related gastroenteritis.[1]

Enterococci are benign bacteria when they reside in their normal habitat such as the gastrointestinal tracts of human or animals. Outside of their normal habitat, *Enterococci* are pathogenic causing urinary tract and wound infections, and life-threatening diseases such as bacteraemia, endocarditis, and meningitis. *Enterococci* easily colonize open wounds and skin ulcers.

Compounding their pathogenesis, *Enterococci* are also some of the most antibiotic resistant bacteria, particularly from human sources. Studies have shown that certain strains of *Enterococci* are resistant to expensive and potent antibiotics such as vancomycin. This is particularly worrisome for the medical community since these antibiotics are given as a last resort to fight severe bacterial infections.

Several intrinsic features of the *Enterococcus* genus allow it to survive for extended periods of time, leading to its extended survivability and diffusion. For example, *Enterococci* have been shown to survive for 30 minutes at 60°C and persist in the presence of detergents. As such, the inherent ruggedness of *Enterococcus* confers it a strong tolerance to many classes of antibiotics.

The Human Enterococcus ID™ service is designed around the principle that certain strains of the *Enterococcus* genus are specific to humans.[2,3,4] These *Enterococci* can be used as indicators of human fecal contamination. Strains of *Enterococcus faecium*, *Enterococcus faecalis* and yellow-pigmented *Enterococci* have been shown to be from human sources.[2,3,4] Within these *Enterococcus spp.* are genes associated with *Enterococci* that are specific to humans.[5] The Human Enterococcus ID™ service targets the esp human gene biomarker in *Enterococcus faecium*.[6]

One of the advantages of the Human Enterococcus ID™ service is that the entire population of *Enterococci* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish. It has been shown that if the total *Enterococci* count (irrespective of the volume of water) of the sample is equal to or greater than 100, the reliability of the analysis is greater, particularly in regards to negative results.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection by gel electrophoresis.

The gel electrophoresis apparatus uses an electrical field to distinguish different DNA fragments according to their molecular weights. Lighter DNA fragments will move farther along the gel than their heavier counterparts. At the end of the procedure different bands of accumulated DNA fragments will aggregate at different parts of the gel. It is this accumulation of DNA fragments that creates a band on the gel. Researchers use these bands to distinguish certain genomes such as the human gene biomarker from *Enterococcus faecium.*

These banding patterns confirm or negate the presence of the *Enterococci* human gene biomarker. As such, the banding patterns provide a reliable indicator of human fecal contamination. To strengthen the validity of the results, the Human Enterococcus ID™ service should be combined with other DNA analytical services such as the Human Bacteroidetes ID™ and Human Fecal Virus ID™ services.

[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Wheeler, A.L., P.G. Hartel, D.G. Godfrey, J.L. Hill, and Segars W.I. 2002. **Potential of *Enterococcus faecalis* as a human fecal indicator for microbial source tracking.** J Environ Qual. 31(4):1286-93.

[3] Bahirathan ML, Puente L, Seyfried P. 1998. **Use of yellow-pigmented enterococci as a specific indicator of human and nonhuman sources of faecal pollution.** Can J Microbiol 44:1066-1071.

[4] Quednau, M., Ahrne, S., Molin, G. **Genomic Relationships between Enterococcus faecium Strains from Different Sources and with Different Antibiotic Resistance Profiles Evaluated by Restriction Endonuclease Analysis of Total Chromosomal DNA Using EcoRI and PvuII.** Appl. Environ. Microbiol. 1999 65: 1777-1780.

[5] Hammerum, A.M., and L.B. Jensen. 2002. **Prevalence of esp, encoding the enterococcal surface protein, in *Enterococcus faecalis* and *Enterococcus faecium* isolates from hospital patients, poultry, and pigs in Denmark.** J. Clin. Microbiol. 40: 4396.

[6] Scott, T.M., T.M. Jenkins, J. Lukasik, and J.B. Rose. 2005. **Potential Use of a Host Associated Molecular Marker in *Enterococcus faecium* as an Index of Human Fecal Pollution.** Environ. Sci. Technol. 39: 283-287.

[7] **EPA Method 1600: Membrane Filter Test Method for Enterococci In Water (1997).**

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CHAIN OF CUSTODY RECORD

SAMPLE SITE:_____ TRACKING CODE:_____

SAMPLER:_____ WITNESS:_____

Sample ID	Media	Sample Date	Sample Time	# of Containers	Analysis

Samples Delivered By	Samples Received By	Date	Time	Lab Numbers

Explanation of Terms Used in Chain-of-Custody Record

Sample Site:	The name of the site being sampled.
Tracking Code:	The resource tracking code for the project.
Sampler:	The name of the individual(s) performing the sampling.
Witness:	The name of any witnesses to the sampling event, if so desired or required.
Sample ID:	The specific sample identification number, unique to each sample set.
Media:	The sample media (e.g. ground water).
Sample Date:	The date on which the sample was taken, including month, day, and year.
Sample Time:	The time at which the sample was taken, using military time (e.g. 9:40 AM would be 0940; 2:15 PM would be 1415).
# of Containers:	The number of containers for that particular sample set.
Analysis:	The type(s) of analysis required for that particular sample or sample set. NOTE: Confer with a laboratory representative to ensure that the analysis you are requesting is clearly understood and that the lab is capable of performing those analyses.
Samples Delivered By:	The signature of the individual who is delivering the samples to the laboratory. Under most circumstances, this will be one of the individuals who performed the sampling.
Samples Received By:	The signature of the individual who receives the samples for the laboratory.
Date:	The date on which the samples are delivered to the lab, including month, day, and year.
Time:	The time at which the samples are delivered to the laboratory, in military time.
Lab Numbers:	The number(s) that the laboratory assigns to the samples for internal tracking purposes.

APPENDIX E

DNA Sample Site Locations

The sites listed below will be sampled for DNA. The Ecorse River sites will be sampled up to 2 times during dry weather and up to 1 time during wet weather. The Detroit River sites will also be sampled up to 1 time during wet weather.

- EC1
- EC2
- EC3
- EC4
- EC5
- EC6
- EC7
- EC8
- EC10
- DR3
- DR4
- DR5
- DR6

Appendix C – *E. coli* Analytical Data

DAILY GEOMETRIC MEAN VALUES (CFU/100ML)

Sample Site	05/01/07	05/08/07	05/15/07	05/22/07	05/29/07	06/05/07	06/12/07	06/19/07	06/26/07	07/03/07	07/10/07	07/17/07	07/24/07	07/31/07	08/07/07	08/14/07	08/21/07	08/28/07	09/04/07	09/11/07	09/18/07	09/25/07	10/02/07
Detroit River																							
DR0 (A-INT)	27	10	10	10	10	10	10	14	10	10	10	14	13	10	19	13	24	10	10	30	10	10	24
DR1 (A-C)	10	10	10	10	10	22	10	10	10	10	10	10	10	22	14	14	14	10	10	13	97	10	23
DR2 (A-C)	28	10	10	10	10	10	13	14	10	10	14	14	16	24	68	46	50	10	13	107	10	10	24
DR3 (A-C)	16	10	10	13	10	17	10	17	10	10	10	10	10	10	25	10	36	28	10	125	14	18	80
DR4 (A-C)	14	10	10	10	10	10	10	10	10	10	10	10	13	10	31	24	28	10	13	130	10	10	51
DR5 (A-INT)	73	16	18	18	19	39	14	42	28	29	17	13	18	18	24	23	262	31	13	219	34	18	**391**
DR6 (A-E)	34	18	19	19	11	21	16	22	32	35	28	44	23	14	108	66	**2,125**	55	23	171	29	66	**491**
DR7 (A-C)	84	14	13	18	13	207	10	20	16	20	19	14	13	13	179	52	**7,027**	62	14	77	37	14	**487**
DR7 (D-G)	10	10	19	10	10	29	10	10	10	10	12	12	10	10	12	12	**2,584**	10	10	10	14	13	150
DR8 (B-F)	16	10	10	10	11	36	11	10	10	12	11	12	10	10	24	12	**6,312**	21	16	13	23	14	40
Ecorse River																							
EC1	**4,017**	**365**	**949**	184	**726**	**2,561**	**814**	**4,328**	**602**	**2,088**	**1,305**	**12,132**	**1,951**	**1,598**	**21,828**	**647**	**1,881**	**917**	136	**9,987**	**323**	**691**	**13,662**
EC2	**1,876**	**788**	**6,516**	**5,261**	**982**	**4,552**		**8,157**		**7,892**		**108,774**		**2,190**	**54,514**		**5,192**	**10,585**		22	**375**		**27,589**
EC3	**1,274**	**1,651**	**1,279**	191	**346**	**1,895**	**1,375**	**5,013**	**3,982**	**3,577**	**8,909**	**12,500**	**1,624**	**1,127**	**13,954**	**1,887**	**5,004**	221	**862**	**15,715**	**857**	**1,119**	**7,953**
EC4	**4,167**	**306**	**2,257**	159	207	**1,174**	**3,248**	**7,450**	**2,290**				239		**30,330**		**3,917**	**405**	62	**8,812**	**1,483**		**4,463**
EC5	**4,482**	**3,342**	**4,088**	**6,172**		**7,013**		**4,579**	**1,437**	**31,748**	**10,811**	**13,351**	**22,894**	**5,601**	**105,762**	**52,415**	**6,380**	**13,635**	**6,753**	**23,380**	**20,448**	**7,556**	**2,010**
EC6	**3,439**	**446**	**1,993**	**460**	**1,715**	**2,026**	**2,893**	**2,280**	**3,284**	**2,631**	**1,011**	**10,379**	**2,932**	**3,583**	**15,874**	**2,695**	**4,294**	**1,756**	**1,282**	17	**1,476**	**1,009**	**6,207**
EC7	**3,139**	148	**947**	**678**	**810**	**2,154**	**1,461**	**5,084**	**1,843**	**3,208**	**609**	**2,932**	**952**	**2,596**	**19,480**	**2,289**	**4,277**	**878**	**765**	**3,989**	**379**	**698**	**13,208**

Notes: indicates exceedence of the daily water quality standard of 300 cfu/100mL

MONTHLY GEOMETRIC MEAN VALUES (CFU/100ML)

Sample Site	05/29/07	06/05/07	06/12/07	06/19/07	06/26/07	07/03/07	07/10/07	07/17/07	07/24/07	07/31/07	08/07/07	08/14/07	08/21/07	08/28/07	09/04/07	09/11/07	09/18/07	09/25/07	10/02/07
Detroit River																			
DR0 (A-INT)	12	10	10	11	11	11	11	11	11	11	13	13	15	14	14	16	15	12	15
DR1 (A-C)	10	12	12	12	12	12	10	10	10	12	13	14	15	15	12	12	18	16	19
DR2 (A-C)	12	10	10	11	11	11	12	12	13	15	22	28	36	33	29	32	23	17	20
DR3 (A-C)	11	12	12	13	12	12	11	11	10	10	12	12	16	19	19	26	28	25	30
DR4 (A-C)	11	10	10	10	10	10	10	10	10	10	13	16	19	18	19	26	21	17	24
DR5 (A-INT)	24	21	20	24	26	28	24	23	20	18	18	19	34	38	35	55	60	35	58
DR6 (A-E)	19	17	17	17	19	24	26	31	32	27	34	40	87	104	114	125	106	53	82
DR7 (A-C)	20	24	23	25	24	27	16	18	16	15	24	29	101	**139**	**143**	121	113	33	49
DR7 (D-G)	11	14	14	12	12	12	10	11	11	11	11	11	33	33	33	31	33	11	19
DR8 (B-F)	11	13	14	14	14	14	11	11	11	11	13	13	45	52	58	51	58	17	20
Ecorse River																			
EC1	**714**	**653**	**766**	**1,038**	**1,316**	**1,626**	**1,421**	**2,438**	**2,079**	**2,527**	**4,041**	**3,512**	**2,419**	**2,080**	**1,270**	**1,086**	**945**	**774**	**1,328**
EC2	**2,185**	**2,609**																	
EC3	**708**	**767**	**739**	**971**	**1,783**	**2,844**	**3,876**	**6,027**	**4,811**	**3,737**	**4,906**	**3,597**	**2,995**	**2,010**	**1,905**	**1,951**	**1,666**	**1,235**	**2,529**
EC4	**624**	**485**	**777**	**987**	**1,683**												**1,323**		
EC5								**7,868**	**10,856**	**14,250**	**18,128**	**24,857**	**21,445**	**19,333**	**20,071**	**14,841**	**12,294**	**12,717**	**8,672**
EC6	**1,193**	**1,073**	**1,559**	**1,602**	**2,373**	**2,585**	**2,250**	**2,905**	**3,054**	**3,108**	**4,453**	**5,417**	**4,541**	**4,098**	**3,337**	**851**	**754**	**565**	**727**
EC7	**753**	**698**	**1,104**	**1,544**	**1,887**	**2,484**	**1,930**	**2,218**	**1,587**	**1,699**	**2,437**	**3,176**	**3,426**	**3,371**	**2,640**	**1,922**	**1,342**	**934**	**1,605**

Notes: indicates exceedence of the monthly water quality standard of 130 cfu/100mL

RAW E. COLI ANALYTICAL DATA (CFU/100ML)

Station ID	Site*	05/01/07	05/08/07	05/15/07	05/22/07	05/29/07	06/05/07	06/12/07	06/19/07	06/26/07	07/03/07	07/10/07	07/17/07	07/24/07	07/31/07	08/07/07	08/14/07	08/21/07	08/28/07	09/04/07	09/11/07	09/18/07	09/25/07	10/02/07
DR0	A	190	10	10	10	10	10	10	10	10	10	10	10	20	10	70	20	130	10	10	280	10	10	140
	B	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	INT	10	10	10	10	10	10	10	25	10	10	10	30	10	10	10	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	10	40	10	10	10	50	10	10	10	10	10	10	10	10	10	10	10
	D	10	10	10	10	10	30	10	10	80	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	E	10	20	10	10	10	10	30	70	150	10	10	10	10	10	10	10	10	10	10	10	10	20	20
US Daily Geomean (A-INT)		*27*	*10*	*10*	*10*	*10*	*10*	*10*	*14*	*10*	*10*	*10*	*14*	*13*	*10*	*19*	*13*	*24*	*10*	*10*	*30*	*10*	*10*	*24*
CA Daily Geomean (C-D)		*10*	*13*	*10*	*10*	*10*	*14*	*14*	*30*	*49*	*10*	*10*	*17*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*13*	*13*
Transect Daily Geomean		*16*	*11*	*10*	*10*	*10*	*12*	*12*	*20*	*22*	*10*	*10*	*16*	*11*	*10*	*14*	*11*	*15*	*10*	*10*	*17*	*10*	*11*	*17*
DR1	A	10	10	10	10	10	20	10	10	10	10	10	10	10	10	30	30	30	10	10	20	1,800	10	40
	B	10	10	10	10	10	50	10	10	10	10	10	10	10	110	10	10	10	10	10	10	50	10	30
	C	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	INT	10	10	10	10	10	10	10	15	10	10	10	10	10	15	10	10	10	10	10	10	20	15	10
	D	10	10	10	10	10	10	10	20	10	10	10	10	10	20	10	10	10	10	10	10	30	20	10
	E	110	10	10	10	10	280	10	10	10	30	10	20	10	10	30	20	1,000	40	60	1,800	10	10	80
US Daily Geomean (A-C)		*10*	*10*	*10*	*10*	*10*	*22*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*22*	*14*	*14*	*14*	*10*	*10*	*13*	*97*	*10*	*23*
CA Daily Geomean (INT-E)		*22*	*10*	*10*	*10*	*10*	*30*	*10*	*14*	*10*	*14*	*10*	*13*	*10*	*14*	*14*	*13*	*46*	*16*	*18*	*56*	*18*	*14*	*20*
Transect Daily Geomean		*15*	*10*	*10*	*10*	*10*	*26*	*10*	*12*	*10*	*12*	*10*	*11*	*10*	*18*	*14*	*13*	*26*	*13*	*13*	*27*	*42*	*12*	*21*
DR2	A	230	10	10	10	10	10	20	30	10	10	10	30	10	140	70	90	80	10	10	470	10	10	140
	B	10	10	10	10	10	10	10	10	10	10	10	10	40	10	220	110	160	10	20	130	10	10	10
	C	10	10	10	10	10	10	10	10	10	10	30	10	10	10	20	10	10	10	10	20	10	10	10
	D	10	10	10	10	10	10	10	10	20	10	10	10	10	10	10	20	10	30	10	20	10	10	10
	INT	10	10	10	10	10	10	10	10	15	10	10	20	10	25	10	55	10	20	15	15	10	10	10
	E	10	10	10	10	10	10	10	10	10	10	10	30	10	40	10	90	10	10	20	10	10	10	10
	F	30	300	10	10	10	20	10	10	10	10	10	10	10	10	230	10	230	10	10	170	10	10	50
US Daily Geomean (A-C)		*28*	*10*	*10*	*10*	*10*	*10*	*13*	*14*	*10*	*10*	*14*	*14*	*16*	*24*	*68*	*46*	*50*	*10*	*13*	*107*	*10*	*10*	*24*
CA Daily Geomean (INT-F)		*14*	*31*	*10*	*10*	*10*	*13*	*10*	*10*	*11*	*10*	*10*	*18*	*10*	*22*	*28*	*37*	*28*	*13*	*14*	*29*	*10*	*10*	*17*
Transect Daily Geomean		*18*	*16*	*10*	*10*	*10*	*11*	*11*	*12*	*12*	*10*	*12*	*15*	*12*	*20*	*35*	*37*	*31*	*13*	*13*	*48*	*10*	*10*	*18*
DR3	A	40	10	10	20	10	50	10	50	10	10	10	10	10	10	160	10	470	220	10	4,900	10	10	430
	B	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	40	30	30	120
	C	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	20	10
	INT	10	10	10	10	10	10	10	10	15	10	10	10	10	10	10	15	35	10	10	10	10	15	10
	D	10	10	10	10	10	10	10	10	20	10	10	10	10	10	10	20	60	10	10	10	10	10	10
	E	560	80	70	10	10	10	30	30	70	100	1,000	270	50	240	2,800	250	800	210	200	490	130	150	450
US Daily Geomean (A-C)		*16*	*10*	*10*	*13*	*10*	*17*	*10*	*17*	*10*	*10*	*10*	*10*	*10*	*10*	*25*	*10*	*36*	*28*	*10*	*125*	*14*	*18*	*80*
CA Daily Geomean (INT-E)		*38*	*20*	*19*	*10*	*10*	*10*	*14*	*14*	*28*	*22*	*46*	*30*	*17*	*29*	*65*	*42*	*119*	*28*	*27*	*37*	*24*	*28*	*36*
Transect Daily Geomean		*25*	*14*	*14*	*11*	*10*	*13*	*12*	*16*	*17*	*15*	*22*	*17*	*13*	*17*	*41*	*21*	*65*	*28*	*16*	*68*	*18*	*23*	*53*
DR4	A	30	10	10	10	10	10	10	10	10	10	10	10	10	10	300	140	220	10	20	5,500	10	10	340
	B	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	20	10	10	40
	C	10	10	10	10	10	10	10	10	10	10	10	10	20	10	10	10	10	10	10	20	10	10	10
	INT	10	15	15	10	40	25	10	10	10	10	10	35	55	55	10	30	45	20	30	45	10	10	10
	D	10	20	20	10	70	40	10	10	10	10	10	60	90	100	10	50	80	30	50	70	10	10	10
	E	530	10	70	60	110	30	30	10	210	50	70	170	50	70	2,100	70	1,900	70	60	670	60	50	60
US Daily Geomean (A-C)		*14*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*13*	*10*	*31*	*24*	*28*	*10*	*13*	*130*	*10*	*10*	*51*
CA Daily Geomean (INT-E)		*38*	*14*	*28*	*18*	*68*	*31*	*14*	*10*	*28*	*17*	*19*	*71*	*63*	*73*	*59*	*47*	*190*	*35*	*45*	*128*	*18*	*17*	*18*
Transect Daily Geomean		*23*	*12*	*17*	*13*	*26*	*18*	*12*	*10*	*17*	*13*	*14*	*27*	*28*	*27*	*43*	*34*	*73*	*19*	*24*	*129*	*13*	*13*	*31*
DR5	A	3,900	40	60	60	70	600	30	20	210	40	50	20	40	60	130	20	120,000	290	20	30,000	130	30	100,000
	B	10	10	10	10	10	10	10	80	10	30	10	10	10	10	10	30	10	10	10	10	20	10	30
	INT	10	10	10	10	10	10	10	45	10	20	10	10	15	10	10	20	15	10	10	35	15	20	20
	C	10	10	10	10	10	10	10	10	10	10	10	10	20	10	10	10	20	10	10	60	10	30	10
	D	200	10	30	10	10	10	30	10	10	30	40	40	20	10	2,200	100	1,800	10	300	2,100	10	590	10
	E	2,900	60	3,100	40	70	110	60	110	60	50	450	110	60	50	9,400	650	2,500	510	4,300	8,300	9,000	7,800	880
US Daily Geomean (A-INT)		*73*	*16*	*18*	*18*	*19*	*39*	*14*	*42*	*28*	*29*	*17*	*13*	*18*	*18*	*24*	*23*	*262*	*31*	*13*	*219*	*34*	*18*	*391*
CA Daily Geomean (C-E)		*180*	*18*	*98*	*16*	*19*	*22*	*26*	*22*	*18*	*25*	*56*	*35*	*29*	*17*	*591*	*87*	*448*	*37*	*235*	*1,015*	*97*	*517*	*44*
Transect Daily Geomean		*115*	*17*	*42*	*17*	*19*	*30*	*19*	*30*	*22*	*27*	*31*	*21*	*23*	*18*	*118*	*45*	*343*	*34*	*54*	*471*	*57*	*97*	*132*

RAW E. COLI ANALYTICAL DATA (CFU/100ML)

Station ID	Site*	05/01/07	05/08/07	05/15/07	05/22/07	05/29/07	06/05/07	06/12/07	06/19/07	06/26/07	07/03/07	07/10/07	07/17/07	07/24/07	07/31/07	08/07/07	08/14/07	08/21/07	08/28/07	09/04/07	09/11/07	09/18/07	09/25/07	10/02/07
DR6	A	2,200	90	280	60	10	200	120	470	280	60	920	680	650	30	50,000	1,600	30,000	270	300	70	530	730	7,100
	B	20	20	10	20	10	10	10	10	10	90	20	20	10	20	150	100	17,000	160	10	470	40	10	780
	C	10	10	10	10	10	20	10	10	10	10	10	10	10	10	10	10	6,300	60	10	500	10	10	4,300
	D	10	10	10	10	10	10	10	10	40	10	10	10	10	10	20	10	90	10	20	40	10	80	30
	E	10	10	10	20	20	10	10	10	30	100	10	120	10	10	10	80	150	20	10	220	10	220	40
	F	150	10	10	10	30	10	20	10	30	20	20	30	70	20	370	10	310	10	30	410	410	310	20
	G	1,600	20	10	10	90	140	30	10	70	100	150	40	60	110	2,100	460	800	530	560	5,400	5,100	2,900	420
	H	310	70	10	20	50	100	390	10	10	180	240	90	520	160	230	90	200	190	10	2,400	90	30	11,000
US Daily Geomean (A-E)		*34*	*18*	*19*	*19*	*11*	*21*	*16*	*22*	*32*	*35*	*28*	*44*	*23*	*14*	*108*	*66*	*2,125*	*55*	*23*	*171*	*29*	*66*	*491*
CA Daily Geomean (F-H)		*421*	*24*	*10*	*13*	*51*	*52*	*62*	*10*	*28*	*71*	*90*	*48*	*130*	*71*	*563*	*75*	*367*	*100*	*55*	*1,745*	*573*	*300*	*452*
Transect Daily Geomean		*87*	*20*	*15*	*16*	*20*	*29*	*27*	*16*	*30*	*46*	*44*	*45*	*44*	*26*	*201*	*69*	*1,101*	*69*	*32*	*408*	*89*	*117*	*476*
DR7	A	180	30	20	60	10	310	10	80	40	80	70	10	20	20	510	90	6,300	80	30	170	520	10	480
	B	30	10	10	10	20	120	10	10	10	10	10	30	10	10	160	20	8,100	50	10	90	10	10	750
	C	110	10	10	10	10	240	10	10	10	10	10	10	10	10	70	80	6,800	60	10	30	10	30	320
	D	10	10	10	10	10	110	10	10	10	10	10	20	10	10	20	20	2,600	10	10	10	10	10	10
	E	10	10	10	10	10	30	10	10	10	10	10	10	10	10	10	10	3,400	10	10	10	20	10	340
	F	10	10	60	10	10	20	10	10	10	10	10	10	10	10	10	10	2,800	10	10	10	10	30	390
	G	10	10	20	10	10	10	10	10	10	10	20	10	10	10	10	10	1,800	10	10	10	20	10	380
	INT	10	10	15	10	10	10	10	10	10	10	15	10	10	10	10	10	945	10	10	10	15	305	510
	H	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	90	10	10	10	10	600	640
	I	60	30	10	10	40	160	40	10	70	10	30	50	10	10	10	40	500	160	390	580	1,900	750	310
US Daily Geomean (A-C)		*84*	*14*	*13*	*18*	*13*	*207*	*10*	*20*	*16*	*20*	*19*	*14*	*13*	*13*	*179*	*52*	*7,027*	*62*	*14*	*77*	*37*	*14*	*487*
US Daily Geomean (D-G)		*10*	*10*	*19*	*10*	*10*	*29*	*10*	*10*	*10*	*10*	*12*	*12*	*10*	*10*	*12*	*12*	*2,584*	*10*	*10*	*10*	*14*	*13*	*150*
CA Daily Geomean (INT - I)		*18*	*14*	*11*	*10*	*16*	*25*	*16*	*10*	*19*	*10*	*17*	*17*	*10*	*10*	*10*	*16*	*349*	*25*	*34*	*39*	*66*	*516*	*466*
Transect Daily Geomean		*23*	*12*	*14*	*12*	*12*	*50*	*11*	*12*	*14*	*12*	*15*	*14*	*11*	*11*	*25*	*20*	*1,913*	*23*	*16*	*28*	*30*	*41*	*300*
DR8	A	1,000	10	10	10	10	490	40	10	10	10	50	50	80	40	760	110	3,000	80	100	280	160	150	2,800
	B	110	10	10	10	10	80	20	10	10	30	20	10	10	10	120	30	8,500	100	60	20	320	30	250
	C	10	10	10	10	20	100	10	10	10	10	10	30	10	10	30	10	5,800	10	10	20	20	10	130
	D	10	10	10	10	10	10	10	10	10	10	10	10	10	10	20	10	9,600	20	10	10	10	10	30
	E	10	10	10	10	10	40	10	10	10	10	10	10	10	10	10	10	7,300	20	10	10	10	10	10
	F	10	10	10	10	10	20	10	10	10	10	10	10	10	10	10	10	2,900	10	20	10	10	20	10
	G	10	10	10	10	10	10	10	10	10	10	10	20	10	10	10	10	10	10	10	10	10	10	10
	H	50	10	30	10	10	40	10	10	10	20	10	10	10	10	250	10	220	10	10	10	10	10	10
	I	200	10	10	20	10	120	10	30	30	10	10	20	10	30	260	10	260	10	40	100	40	270	390
US Daily Geomean (B-F)		*16*	*10*	*10*	*10*	*11*	*36*	*11*	*10*	*10*	*12*	*11*	*12*	*10*	*10*	*24*	*12*	*6,312*	*21*	*16*	*13*	*23*	*14*	*40*
CA Daily Geomean (G-I)		*46*	*10*	*14*	*13*	*10*	*36*	*10*	*14*	*14*	*13*	*10*	*16*	*10*	*14*	*87*	*10*	*83*	*10*	*16*	*22*	*16*	*30*	*34*
Transect Daily Geomean		*36*	*10*	*11*	*11*	*11*	*49*	*13*	*11*	*11*	*12*	*13*	*16*	*13*	*13*	*53*	*15*	*1,372*	*19*	*20*	*22*	*25*	*24*	*60*

RAW E. COLI ANALYTICAL DATA (CFU/100ML)

Station ID	Site*	05/01/07	05/08/07	05/15/07	05/22/07	05/29/07	06/05/07	06/12/07	06/19/07	06/26/07	07/03/07	07/10/07	07/17/07	07/24/07	07/31/07	08/07/07	08/14/07	08/21/07	08/28/07	09/04/07	09/11/07	09/18/07	09/25/07	10/02/07
EC0	**A**	2,900					2,500		11,000															
EC1	**A**	4,000	350	500	320	1,500	2,400	900	4,300	750	4,100	950	16,000	3,300	1,600	20,000	520	2,000	700	500	8,300	250	540	8,500
	B	3,600	310	900	390	750	2,500	750	4,600	360	600	1,800	12,000	2,500	1,500	26,000	840	900	1,100	10	12,000	270	610	30,000
	C	4,500	450	1,900	50	340	2,800	800	4,100	810	3,700	1,300	9,300	900	1,700	20,000	620	3,700	1,000	500	10,000	500	1,000	10,000
Daily Geomean		*4,017*	*365*	*949*	*184*	*726*	*2,561*	*814*	*4,328*	*602*	*2,088*	*1,305*	*12,132*	*1,951*	*1,598*	*21,828*	*647*	*1,881*	*917*	*136*	*9,987*	*323*	*691*	*13,662*
EC2	**A**	1,500	510	6,500	5,600	790	5,000		9,500		8,400		90,000		3,500	90,000		7,000	11,000		20	10		70,000
	B	2,200	400	5,600	5,200	1,000	4,100		8,400		7,700		130,000		1,200	60,000		10,000	9,800		50	2,400		10,000
	C	2,000	2,400	7,600	5,000	1,200	4,600		6,800		7,600		110,000		2,500	30,000		2,000	11,000		10	2,200		30,000
Daily Geomean		*1,876*	*788*	*6,516*	*5,261*	*982*	*4,552*		*8,157*		*7,892*		*108,774*		*2,190*	*54,514*		*5,192*	*10,585*		*22*	*375*		*27,589*
EC3	**A**	2,300	2,000	1,900	500	140	1,700	2,600	300	5,500	4,400	13,000	15,000	1,700	1,000	19,000	2,400	3,900	40	1,600	18,000	900	1,500	13,000
	B	1,000	1,500	1,000	40	800	2,500	1,000	14,000	4,100	5,200	8,000	14,000	1,200	1,100	13,000	2,000	5,100	490	500	9,800	700	1,100	8,600
	C	900	1,500	1,100	350	370	1,600	1,000	30,000	2,800	2,000	6,800	9,300	2,100	1,300	11,000	1,400	6,300	550	800	22,000	1,000	850	4,500
Daily Geomean		*1,274*	*1,651*	*1,279*	*191*	*346*	*1,895*	*1,375*	*5,013*	*3,982*	*3,577*	*8,909*	*12,500*	*1,624*	*1,127*	*13,954*	*1,887*	*5,004*	*221*	*862*	*15,715*	*857*	*1,119*	*7,953*
EC4	**A**	5,300	280	2,300	500	360	1,500	2,800	5,700	3,300				200	-	30,000	-	3,700	260	270	13,000	1,200		4,300
	B	3,900	540	2,500	160	310	1,100	1,800	9,300	2,600				400	-	30,000	-	5,800	910	90	9,400	1,700		5,300
	C	3,500	190	2,000	50	80	980	6,800	7,800	1,400				170	-	31,000	-	2,800	280	10	5,600	1,600		3,900
Daily Geomean		*4,167*	*306*	*2,257*	*159*	*207*	*1,174*	*3,248*	*7,450*	*2,290*				*239*	-	*30,330*	-	*3,917*	*405*	*62*	*8,812*	*1,483*		*4,463*
EC5	**A**	4,100	2,900	4,900	4,600		4,900		12,000	30	40,000	12,000	14,000	40,000	6,400	130,000	40,000	5,900	13,000	2,800	60,000	9,500	5,300	500
	B	6,100	3,300	3,400	7,000		6,400		10,000	9,000	40,000	13,000	10,000	30,000	6,100	70,000	60,000	7,100	13,000	10,000	30,000	10,000	7,400	5,800
	C	3,600	3,900	4,100	7,300		11,000		800	11,000	20,000	8,100	17,000	10,000	4,500	130,000	60,000	6,200	15,000	11,000	7,100	90,000	11,000	2,800
Daily Geomean		*4,482*	*3,342*	*4,088*	*6,172*		*7,013*		*4,579*	*1,437*	*31,748*	*10,811*	*13,351*	*22,894*	*5,601*	*105,762*	*52,415*	*6,380*	*13,635*	*6,753*	*23,380*	*20,448*	*7,556*	*2,010*
EC6	**A**	3,300	600	2,400	510	1,600	2,000	2,700	2,600	3,300	2,300	970	10,000	3,500	3,400	40,000	2,700	6,000	1,500	1,300	10	2,700	850	7,000
	B	4,400	380	1,500	360	1,500	1,600	2,300	1,900	3,700	2,200	820	13,000	2,000	3,300	10,000	2,500	5,500	1,900	1,800	50	700	1,100	5,100
	C	2,800	390	2,200	530	2,100	2,600	3,900	2,400	2,900	3,600	1,300	8,600	3,600	4,100	10,000	2,900	2,400	1,900	900	10	1,700	1,100	6,700
Daily Geomean		*3,439*	*446*	*1,993*	*460*	*1,715*	*2,026*	*2,893*	*2,280*	*3,284*	*2,631*	*1,011*	*10,379*	*2,932*	*3,583*	*15,874*	*2,695*	*4,294*	*1,756*	*1,282*	*17*	*1,476*	*1,009*	*6,207*
EC7	**A**	3,500	30	920	680	720	2,800	1,200	7,300	2,900	2,300	7,800	1,900	300	2,500	21,000	2,000	6,500	770	700	4,400	490	930	12,000
	B	2,600	120	1,100	610	770	1,700	2,000	1,200	1,200	4,100	10	3,400	1,200	2,500	22,000	2,000	2,800	1,100	800	3,700	300	690	16,000
	C	3,400	900	840	750	960	2,100	1,300	15,000	1,800	3,500	2,900	3,900	2,400	2,800	16,000	3,000	4,300	800	800	3,900	370	530	12,000
Daily Geomean		*3,139*	*148*	*947*	*678*	*810*	*2,154*	*1,461*	*5,084*	*1,843*	*3,208*	*609*	*2,932*	*952*	*2,596*	*19,480*	*2,289*	*4,277*	*878*	*765*	*3,989*	*379*	*698*	*13,208*
EC8	**A**	4,900	2,600	5,000	10,000	1,100	4,900	180,000	7,600	2,800	1,700	21,000	3,500	2,900	5,900	80,000	13,000	8,900	4,200	50,000	30,000	30,000	8,700	30,000
	B	5,200	50,000	130,000	90,000	18,000	7,700	170,000	280,000															
EC9	**A**	4,400					7,000		700				4,800	5,500		70,000		8,800			21,000			10,000
EC10	**A**								4,000	3,300	30,000	6,100	6,500	3,300	2,000	40,000	2,500	8,300	600	2,300	30,000	15,000	420	12,000
	B								30,000	60,000	10,000	3,600	6,400	2,200	140,000	90,000	360,000	30,000	40,000	590,000	10,000			9,200
		0.9" rain					**0.5" rain**		**0.2" rain**							**1.6" rain**		**2.4" rain**			**0.6" rain**			**0.8" rain**

Notes: *Sampling sites identified as "INT" indicate the values are interpolated based on the neighboring results.

= Wet weather events. Rainfall volumes were measured at Detroit Metropolitan Airport within 30 hrs of sample collection

= Samples analyzed outside of holding time

= Corresponding duplicate or blank sample outside of precision or accuracy range

Appendix D – Blank and Duplicate *E. coli* Analytical Data

Blank and Duplicate Analytical Results

Week	Blank Sample ID	Blank Sample Result		Corresponding Regular Sample ID	Corresponding Regular Sample Result		Duplicate Sample Result		Comments
1				DR2B01d (24)	<	10	<	10	
	DR3B01b (40)	<	10	DR3B01d (39)	<	10		20	
				DR4E01d (54)		530		390	
	DR6D01b (67)	<	10	DR6D01d (68)		10	<	10	
				DR7F01d (91)	<	10		30	
				DR8D01d (81)	<	10		10	
				EC6A01d (17)		3,300		2,500	
	EC8B01b (45)	<	10	EC8B01d (44)		5,200		4,800	
2				DR1A02-d	<	10	<	10	
	DR3B02-b	<	10	DR3B02-d	<	10	<	10	
				DR6A02-d		90		50	
	DR7C02-b		20	DR7C02-d	<	10		10	Blank outside of the acceptable range
				DR7I02-d		30		40	
	EC2C02-b		10	EC2C02-d		2,400		50	Blank outside of the acceptable range
				EC3B02-d		1,500		1,800	
3				DR0A03-d	<	10	<	10	
	DR2A03-b		110	DR2A03-d	<	10	<	10	Blank outside of the acceptable range
				DR3A03-d	<	10		20	
	DR5A03-b	<	10	DR5A03-d		60		80	
				DR7B03-d		10		20	
	DR8I03-b	<	10	DR8I03-d		10	<	10	
	EC2A03-b	<	10	EC2A03-d		6,500		9,600	
				EC4C03-d		2,000		1,100	
4				DR1C04-d	<	10	<	10	
	DR3D04-b	<	10	DR3D04-d		10		10	
				DR5D04-d		10		10	
	DR6F04-b	<	10	DR6F04-d	<	10		20	
				DR7A04-d		60		40	
	EC2B04-b	<	10	EC2B04-d		5,200		3,700	
				EC5B04-d		7,000		5,600	
5				DR1E05-d		10		10	
	DR2F05-b	<	10	DR2F05-d	<	10	<	10	
				DR4E05-d		110		160	
	DR6E05-b	<	10	DR6E05-d		20		10	
				DR7C05-d	<	10	<	10	
	DR8H05-b	<	10	DR8H05-d		10		10	
	EC2A05-b	<	10	EC2A05-d		790		930	
6				DR1A06-d		20		20	
	DR3B06-b	<	10	DR3B06-d		10		20	
				DR5B06-d		10		40	
	DR6H06-b	<	10	DR6H06-d		100		160	
				DR8A06-d		490		290	
				EC1B06-d		2,500		2,600	
	EC4C06-b	<	10	EC4C06-d		980		1,200	
				EC8A06-d		4,900		5,200	
7				DR1C07-d	<	10	<	10	
	DR3D07-b	<	10	DR3D07-d	<	10	<	10	
				DR5D07-d		30		20	
	DR6G07-b	<	10	DR6G07-d		30		90	
				DR7I07-d		40	<	10	
				EC1A07-d		900		1,300	
	EC4B07-b	<	10	EC4B07-d		1,800		2,400	
				EC6C07-d		3,900		3,200	

Blank and Duplicate Analytical Results

Week	Blank Sample ID	Blank Sample Result		Corresponding Regular Sample ID	Corresponding Regular Sample Result		Duplicate Sample Result		Comments
8				DR1D08-d		20	<	10	
	DR3E08-b	<	10	DR3E08-d		30		10	
				DR5E08-d		110		290	
	DR6F08-b	<	10	DR6F08-d	<	10		20	
	DR7H08-b	<	10	DR7H08-d	<	10		10	
				DR8I08-d		30		40	
				EC1A08-d		4,300		3,600	
				EC4B08-d		9,300		7,600	
				EC7C08-d		15,000		14,000	
9				DR1E09-d	<	10		10	
	DR2F09-b	<	10	DR2F09-d	<	10	<	10	
				DR4E09-d		210		130	
	DR6E09-b	<	10	DR6E09-d		30		20	
	DR7H09-b	<	10	DR7H09-d	<	10	<	10	
				DR8D09-d	<	10	<	10	
	EC4A09-b	<	10	EC4A09-d		3,300		3,600	
				EC7B09-d		1,200		1,200	
10				DR0B10-d	<	10	<	10	
	DR2B10-b	<	10	DR2B10-d	<	10	<	10	
				DR4A10-d	<	10		10	
	DR6H10-b	<	10	DR6H10-d		180		150	
				DR7C10-d	<	10	<	10	
	DR7I10-b	<	10	DR7I10-d	<	10		10	
	EC3C10-b	<	10	EC3C10-d		2,000		4,900	
				EC7A10-d		2,300		2,100	
11				DR0A11-d	<	10	<	10	
	DR2A11-b	<	10	DR2A11-d	<	10		10	
				DR3A11-d	<	10	<	10	
	DR5A11-b	<	10	DR5A11-d		50		20	
				DR7B11-d		10	<	10	
	DR8I11-b	<	10	DR8I11-d	<	10	<	10	
	EC3B11-b	<	10	EC3B11-d		8,000		11,000	
				EC6C11-d		1,300		110	Duplicate outside of the acceptable range
12	DR1A12-b	<	10	DR1A12-d	<	10		10	
				DR3B12-d	<	10	<	10	
	DR5B12-b	<	10	DR5B12-d	<	10		10	
				DR7A12-d		10		20	
	DR8H12-b	<	10	DR8H12-d		10		20	
	EC3A12-b	<	10	EC3A12-d		15,000		15,000	
				EC6B12-d		13,000		13,000	
13	DR1B13-b	<	10	DR1B13-d	<	10	<	10	
				DR3C13-d	<	10		30	
	DR5C13-b	<	10	DR5C13-d		20		20	
				DR6A13-d		650		520	
	DR8G13-b	<	10	DR8G13-d	<	10	<	10	
				EC6A13-d		3,500		2,900	
14	DR1C14-b	<	10	DR1C14-d	<	10	<	10	
				DR3D14-d	<	10	<	10	
	DR5D14-b	<	10	DR5D14-d		10		30	
				DR6B14-d		20		10	
	DR8F14-b	<	10	DR8F14-d	<	10	<	10	
	EC10B14-b	<	10	EC10B14-d		140,000		220,000	
	EC2B14-b	<	10	EC2B14-d		1,200		1,900	
				EC5C14-d		4,500		5,200	

Blank and Duplicate Analytical Results

Week	Blank Sample ID	Blank Sample Result		Corresponding Regular Sample ID	Corresponding Regular Sample Result		Duplicate Sample Result		Comments
15	DR1D15-b	<	10	DR1D15-d	<	10	<	10	
				DR3E15-d		2,800		2,300	
	DR5E15-b	<	10	DR5E15-d		9,400		10,000	
				DR6C15-d		10		10	
				DR7D15-d		20		50	
	DR8E15-b	<	10	DR8E15-d	<	10		30	
				EC10B15-d		90,000		130,000	
				EC4A15-d		30,000		30,000	
	EC6B15-b	<	10	EC6B15-d		10,000		140,000	
16	DR1E16-b	<	10	DR1E16-d		20		40	
				DR2F16-d	<	10		20	
	DR4E16-b	<	10	DR4E16-d		70		40	
				DR6D16-d		10	<	10	
				DR7E16-d		10		30	
	DR8D16-b	<	10	DR8D16-d	<	10		10	
	EC1A16-b	<	10	EC1A16-d		520		570	
				EC3A16-d		2,400		1,900	
17	DR0E17-b	<	10	DR0E17-d	<	10	<	10	
				DR2E17-d	<	10		20	
	DR4D17-b	<	10	DR4D17-d		80		290	
				DR6E17-d		150		170	
				DR7F17-d		2,800		2,500	
	DR8C17-b	<	10	DR8C17-d		5,800		7,100	
				EC4B17-d		5,800		4,100	
				EC5A17-d		5,900		6,400	
	EC6A17-b	<	10	EC6A17-d		6,000		4,300	
18	DR0D18-b	<	10	DR0D18-d	<	10	<	10	
				DR2D18-d		30	<	10	
	DR4C18-b	<	10	DR4C18-d	<	10	<	10	
				DR6F18-d		10		20	
				DR7G18-d	<	10		10	
	DR8B18-b	<	10	DR8B18-d		100		100	
	EC2C18-b	<	10	EC2C18-d		11,000		10,000	
				EC3B18-d		490		600	
19	DR0C19-b	<	10	DR0C19-d	<	10	<	10	
				DR2C19-d	<	10		20	
	DR4B19-b	<	10	DR4B19-d		10		10	
				DR6G19-d		560		290	
				DR7H19-d	<	10	<	10	
	DR8A19-b	<	10	DR8A19-d		100		60	
	EC1C19-b	<	10	EC1C19-d		500		420	
				EC4C19-d	<	10		700	
20	DR0B20-b	<	10	DR0B20-d	<	10	<	10	
				DR2B20-d		130		110	
	DR4A20-b	<	10	DR4A20-d		5,500		5,200	
				DR6H20-d		2,400		1,700	
	DR7C20-b	<	10	DR7C20-d		30		40	
				DR7I20-d		580		1,200	
	EC1A20-b	<	10	EC1A20-d		8,300		7,200	Duplicate samples analyzed outside of holding time
				EC3C20-d		22,000		19,000	
21	DR0A21-b	<	10	DR0A21-d		10		10	
				DR2A21-d	<	10		10	
	DR3A21-b	<	10	DR3A21-d	<	10	<	10	
				DR5A21-d		130		100	
	DR7B21-b	<	10	DR7B21-d		10	<	10	
				DR8I21-d		40		30	
	EC1B21-b	<	10	EC1B21-d		270		160	
				EC7A21-d		490		610	

Blank and Duplicate Analytical Results

Week	Blank Sample ID	Blank Sample Result		Corresponding Regular Sample ID	Corresponding Regular Sample Result		Duplicate Sample Result		Comments
22				DR1A22-d		10	<	10	
	DR3B22-b	<	10	DR3B22-d		30		40	
				DR5B22-d	<	10	<	10	
	DR6A22-b	<	10	DR6A22-d		730		590	
	DR7A22-b	<	10	DR7A22-d		10		10	
				DR8H22-d	<	10		10	
	EC1C22-b	<	10	EC1C22-d		540		900	
				EC3B22-d		1,100		980	
23				DR1B23-d		30		20	
	DR3C23-b	<	10	DR3C23-d	<	10	<	10	
				DR5D23-d		10	<	10	
	DR6B23-b	<	10	DR6B23-d		780		1,600	
				DR7G23-d		380		340	
	DR8B23-b	<	10	DR8B23-d		250		280	
	EC10A23-b	<	10	EC10A23-d		12,000		1,100	
				EC6A23-d		7,000		3,900	

= Samples whose values are less than three time the detection limit.

Appendix E – BST Analytical Data

Monitoring Location	Site	Dry Weather				Wet Weather			
		E. coli Conc. (cfu/100mL)	Enterococcus Conc. (cfu/100mL)	Human Enterococcus Marker (+/-)	Human Bacteroidetes Marker (+/-)	*E. coli* Conc. (cfu/100mL)	Enterococcus Conc. (cfu/100mL)	Human Enterococcus Marker (+/-)	Human Bacteroidetes Marker (+/-)
DR3	E	Not Sampled				800	146	+	-
DR4	E	Not Sampled				1,900	TNTC	+	-
DR5	E	Not Sampled				2,500	89	-	-
DR6	G	Not Sampled				800	108	-	-
EC1	B	10	TNTC	+	-	900	TNTC	-	-
EC2	B	2,400	TNTC	+	-	10,000	TNTC	-	-
EC3	A A B	2,400 1,600	TNTC TNTC	- +	- +	5,100	TNTC	-	-
EC4	B C	90	TNTC	+	-	2,800	TNTC	-	-
EC5	B C	60,000	TNTC	+	+	6,200	TNTC	-	-
EC6	A B C	1,300 2,900	TNTC TNTC	+ -	- -	5,500	TNTC	-	-
EC7	A B B C	930 690 800	132 158 TNTC	- - -	- - -	6,500 16,000 12,000	TNTC TNTC TNTC	- + +	- + +
EC8	A A	50,000 8,700	146 118	- +	- +	8,900	TNTC	+	+
EC10	A B	2,300 590,000	TNTC TNTC	+ +	+ +	8,300 30,000	TNTC TNTC	- +	- +

TNTC = Colonies were to numerous to count.

Attachment B
Detroit River Flow Duration Curve and Time Series Data

Figure B1. Estimated flow duration curve for the Detroit River. Daily flow estimates obtained from the USACE Detroit District for the period 1/1/1977 to 12/31/2007

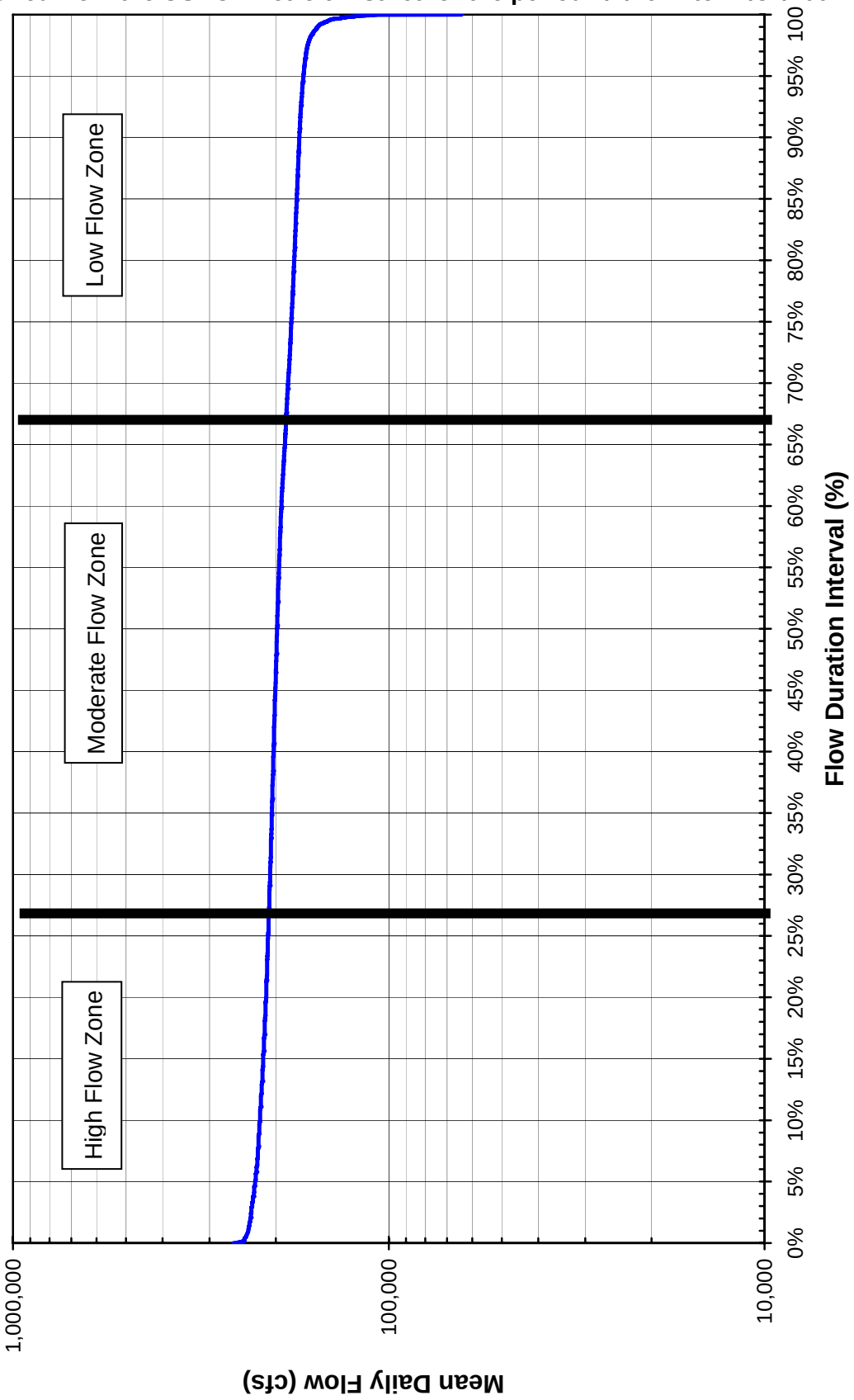
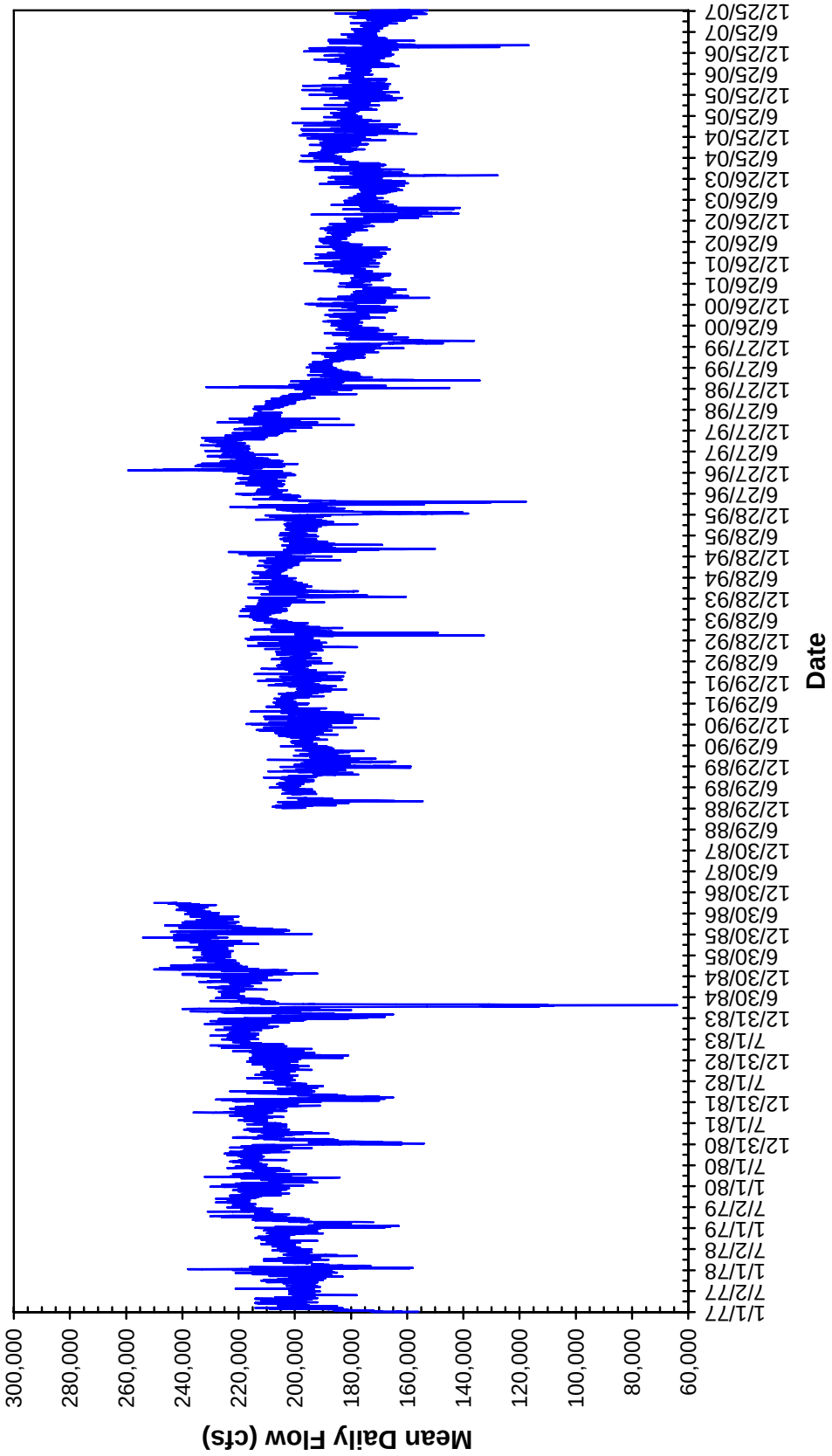


Figure B2. Estimated flow time series for the Detroit River. Daily flow estimates obtained from the USACE Detroit District for the period 1/1/1977 to 12/31/2007



Attachment C
Detroit River NPDES Permits

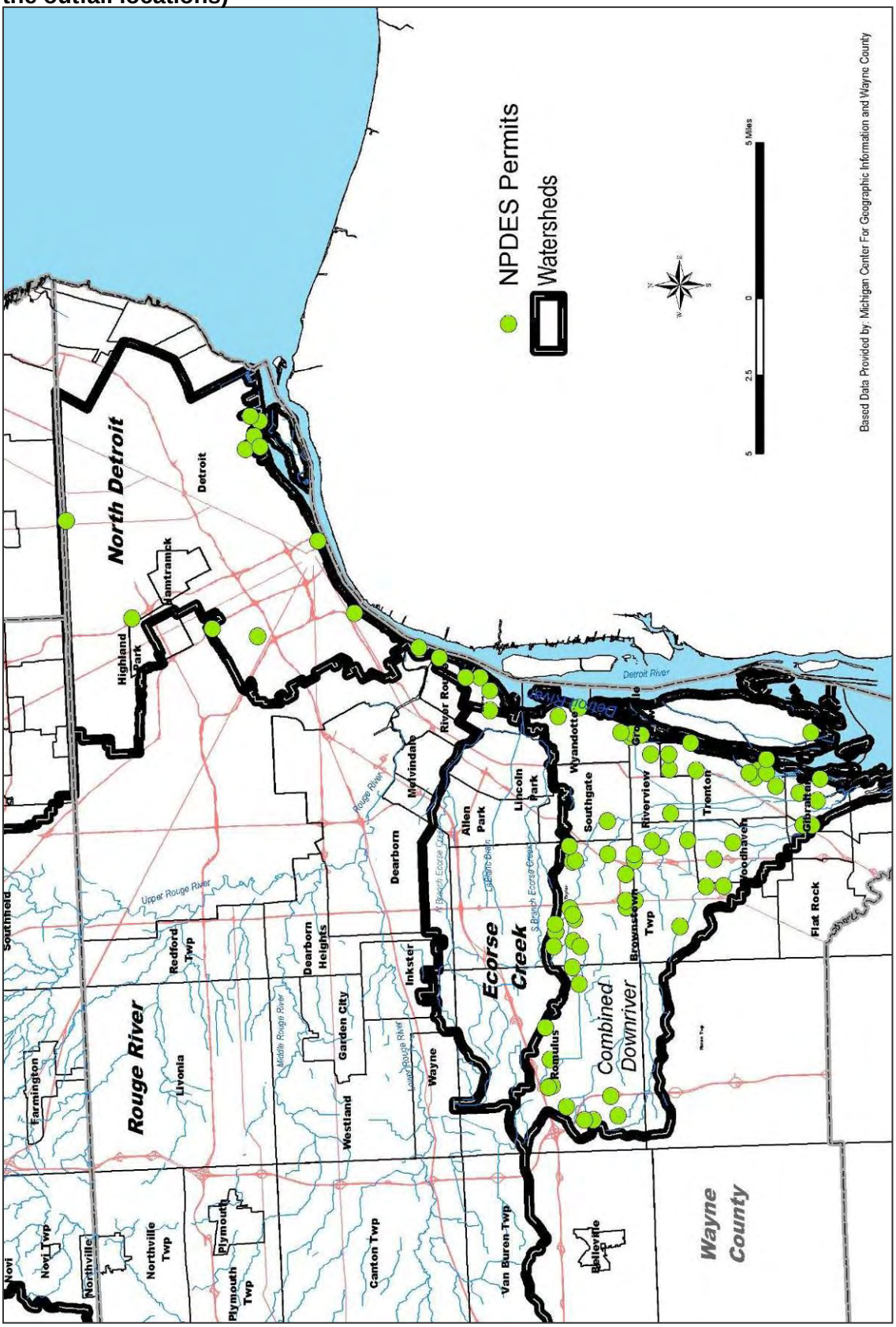
Table C1. NPDES individual permits and Certificates of Coverage in the Combined Downriver and North Detroit portions of the Detroit River Watershed

Permit/ Certificate of Coverage Number	Facility Name
Individual Permits	
MI0000540	BASF-Wyandotte
MI0001724	DECO-River Rouge Plt
MI0001775	DECO-Conners Creek Plt
MI0001791	DECO-Trenton Plt
MI0001953	DECO-Sibley Quarry
MI0002313	US Steel-GL-Ecorse
MI0002381	Taminco-Riverview
MI0002399	DSC-Trenton Plant
MI0003310	Praxair Inc
MI0021156	Wayne Co-Downriver WWTP
MI0021164	Trenton WWTP
MI0022802	Detroit WWTP
MI0026191	Grosse Ile Twp WWTP
MI0026786	US Steel-GL-Zug Island
MI0036072	Southgate/Wyandotte CSO RTF
MI0036846	Detroit Metro Wayne Co Airport
MI0038105	Wyandotte Electric Plant & WFP
MI0043800	S Huron Valley UA WWTP
MI0056243	Steel Rolling Holdings LLC
MI0057359	Ford-Woodhaven Stamping Pt
MI0057364	MDOT State-wide MS4
MI0057709	US Steel-GLW-River Basins
General Permit MIG619000 - Municipal Separate Storm Sewer System (Watershed-Based)	
MIG610017	Romulus MS4-Wayne
MIG610040	Wayne Co MS4
MIG610344	Grosse Ile Twp MS4-Wayne
MIG610345	Riverview MS4-Wayne
MIG610346	Gibraltar MS4-Wayne
MIG610347	Southgate MS4-Wayne
MIG610348	Taylor MS4-Wayne
MIG610349	Wyandotte MS4-Wayne
MIG610352	Ecorse MS-4 Wayne
MIG610353	Huron Charter Twp MS4-Wayne
MIG610354	Woodhaven MS4-Wayne
MIG610355	Sumpter Twp MS4-Wayne
MIG610356	Brownstown Twp MS4-Wayne
MIG610359	Woodhaven-Brownstown PS MS4-Wayne
General Permit MIS040000 - Municipal Separate Storm Sewer System (Six Min. Measures)	
MIS040066	Detroit MS4-Wayne
MIS040073	River Rouge MS4-Wayne
MIS040038	Trenton MS4-Wayne
General Permit MIG640000 - Wastewater Discharge from Municipal Potable Water Supply	
MIG640222	DWSD-Water Works Park II WTP
General Permit MIG670000 - Hydrostatic Pressure Test Water	
MIG670085	Buckeye Terminals-Woodhaven
General Permit MIS210000 - Storm Water from Industrial Activities (2-Yr Cycle Watersheds)	
MIS210782	Levy-Brennan Street Dock
MIS210878	Great Lakes Agg-Highland Park
MIS210886	Dolphin Manufacturing
MIS210888	Superior Materials Plt 2

Permit/ Certificate of Coverage Number	Facility Name
MIS210900	Alken Ziegler-Taylor
MIS210905	Oakwood Custom Coating
MIS210906	Oakwood Metal Fabricating
MIS210910	Jamison Industries Inc
General Permit MIS310000 - Storm Water from Industrial Activities (3-Yr Cycle Watersheds)	
MIS310018	Group B Industries Inc
MIS310040	Control Manufacturing
MIS310041	Best Concrete & Supply
MIS310042	Solutia Inc-Trenton-Plant 2
MIS310050	Hawkins Steel Cartage
MIS310051	Humbug Marina-Gibraltar
MIS310086	Chrysler LLC-Trenton
MIS310090	Levy-Ecorse-Plt 3
MIS310091	Taylor Auto Salvage Inc
MIS310094	Summit Industries-Taylor
MIS310095	Saint-Gobain Abrasives Inc
MIS310143	G & J Cartage Co-Taylor
MIS310146	Fritz Enterprises-Taylor
MIS310147	Fritz Enterprises-Brownstown
MIS310183	FPT Hi-Way
MIS310186	Mich ARNG-Taylor Armory OMS8
MIS310189	Phils Auto Parts-Southgate
MIS310209	Suburban Industries-Gibraltar
MIS310210	Trenton Auto Parts
MIS310211	Levy-Penn Landfill
MIS310212	Ash Stevens-Riverview
MIS310216	Doan Companies-Brownstown Plt
MIS310217	Big 4 Auto Parts
MIS310221	Riverview-Land Preserve
MIS310224	Gregory Boat Company-No 1
MIS310225	Gregory Boat Company-No 2
MIS310238	ET US Holdings LLC
MIS310239	Johnson Controls-Taylor
MIS310273	Kreher Wire Processing-Romulus
MIS310276	Ford-Woodhaven Forging Plt
MIS310284	Elkins Machine & Tool-Romulus
MIS310289	Industrial Fab Inc-Rockwood
MIS310292	Waste Mgt of Mi-Area Disposal
MIS310293	Harbor Hill Marina-Detroit
MIS310294	Trenton Forging Co-Trenton
MIS310315	Millcraft SMS Services-Taylor
MIS310316	Contract Freighters-Taylor
MIS310319	Systrand Manufacturing-Trenton
MIS310387	Aztec Manufacturing-Romulus
MIS310419	Landis Machine Shop-Romulus
MIS310428	Detroit Marine-Detroit River
MIS310470	Federal Screw Works-Romulus
MIS310476	Keans Detroit Yacht Harbor
MIS310508	CTS Engineering-Taylor
MIS310510	UPS-Taylor
MIS310518	Apollo Plating Inc-Taylor
MIS310553	CL Rieckhoff Co Inc
MIS310555	Bucks Oil Co Inc

Permit/ Certificate of Coverage Number	Facility Name
MIS310569	Huron Valley Steel Corp
MIS310599	Prime Industries
General Permit MIS410000 - Storm Water from Industrial Activities (4-Yr Cycle Watersheds)	
MIS410395	Barrett Paving-Romulus
MIS410413	Oakite Products-Romulus
MIS410554	Johnson Controls Inc-Rockwood
General Permit MIS320000 - Storm Water Discharges with Required Monitoring	
MIS320014	Grosse Ile Municipal Airport
MIS320020	Buckeye Terminals-Woodhaven

Figure C1. NPDES Permit Locations (note that the depicted locations are not necessarily the outfall locations)



Attachment D
City of Detroit Uncontrolled CSOs Discharging to the Detroit River

Table D1. City of Detroit Uncontrolled CSOs on the Detroit River Shoreline

Outfall Number	Name and Location	Latitude Longitude
080	Fox Creek Backwater Gates (B01) East Jefferson & Fox Creek	42°22'28" 082°56'27"
004	Fairview (DWF) Pump Station (P28 through P31) Parkview & Detroit River - Emergency only	42°21'20" 082°58'01"
005	McClellan (B03) McClellan & Detroit River	42°21'20" 082°58'02"
006	Fischer (B04) Fischer & Detroit River	42°21'16" 082°59'15"
007	Iroquois (B05) Iroquois & Detroit River	42°21'14" 082°59'21"
008	Helen (B06) Helen & Detroit River	42°20'40" 083°00'06"
009	Mt. Elliott (B07) Mt. Elliott & Detroit River	42°20'24" 083°00'28"
011	Adair (B09) Adair & Detroit River	42°20'16" 083°00'41"
012	Joseph Campau (B10) Joseph Campau & Detroit River	42°10'08" 083°01'02"
014	Dubois (B12) Dubois & Detroit River	42°20'01" 083°01'19”
016	Orleans Relief (B15) Orleans (Eastside of) & Detroit River	42°19'54" 083°01'36"
017	Orleans (B14) Orleans (Westside of) & Detroit River	42°19'53" 083°01'37"
018	Riopelle (B16) Riopelle & Detroit River	42°19'52" 083°01'42"
019	Rivard (B17) Rivard & Detroit River	42°19'48" 083°01'55"
020	Hastings (B18) Schweizer Place & Detroit River	42°19'46" 083°02'03"
021	Randolph (B19) Randolph & Detroit River	42°19'29" 083°02'26"
022	Bates (B20) Bates & Detroit River	42°19'38" 083°02'32"
023	Woodward (B21) Woodward & Detroit River	42°19'37" 083°02'35”
024	Griswold (B22) Griswold & Detroit River	42°19'35" 083°02'28"
025	First-Hamilton (B23) First (extended) & Detroit River	42°19'30" 083°02'57"
026	Third St. (B24) Third St. & Detroit River	42°19'28" 083°03'07"
027	Cabacier (B25) Brooklyn (extended) & Detroit River	42°19'24" 083°03'26"
028	Eleventh St. (B26) Eleventh St. & Detroit River	42°19'17" 083°03'46"

Outfall Number	Name and Location	Latitude Longitude
029	Rosa Parks (B27) Rosa Parks & Detroit River	42°19'13" 083°03'56"
030	Vermont (B28) Vermont(extended) & Detroit River	42°19'06" 083°04'09"
031	Eighteenth St. (B29) Eighteenth St. & Detroit River	42°18'57" 083°04'31"
032	Twenty-First St. (B30) Twenty-First St. & Detroit River	42°18'53" 083°04'31"
033	Twenty-Fourth St. (B31) Twenty-Fourth St. & Detroit River	42°18'47" 083°04'42"
034	West Grand Blvd. (B32) West Grand Blvd. & Detroit River	42°18'41" 083°04'50"
035	Swain (B33) Swain & Detroit River	42°18'35" 083°04'56"
036	Scotten (B34) Scotten & Detroit River	42°18'31" 083°05'02"
037	McKinstry (B35) McKinstry & Detroit River	42°18'19" 083°05'13"
038	Summit-Clark (B36) Summit & Detroit River	42°18'14" 083°05'18"
039	Ferdinand (B37) Ferdinand & Detroit River	42°18'13" 083°05'19"
040	Morrell (B38) Morrell & Detroit River	42°18'10" 083°05'22"
041	Junction (B39) Junction & Detroit River	42°18'07" 083°05'25"
042	Campbell (B40) Campbell & Detroit River	42°18'01" 083°05'30"
043	Dragoon (Livernois Relief) (B41) Dragoon (extended) & Detroit	42°17'49" 083°05'41"
044	Schroeder (B42) Schroeder & West Jefferson	42°17'32" 083°06'00"