Grant number(s): C9-994861-02

The Dix River/ Herrington Reservoir Watershed Implementation Project

PON2 0700004524- Microbial Source Tracking in the Dix River Watershed

PON2 0800010319- Watershed Based Plan

Application title: Dix River Herrington/Reservoir Watershed Implementation Plan Project
Application number: 02-11
Project period: April 1, 2006 – October 31, 2009

Submitted by:
Tony Miller
Third Rock Consultants, LLC
2526 Regency Rd, Suite 180
Lexington, KY 40503

The Energy and Environment Cabinet (EEC) and Third Rock Consultants, LLC do not discriminate on the basis of race, color, national origin, sex, age, religion, or disability. The EEC and Third Rock Consultants, LLC will provide, on request, reasonable accommodations including auxiliary aids and services necessary to afford an individual with a disability an equal opportunity to participate in all services, programs and activities. To request materials in an alternative format, contact the Kentucky Division of Water, 200 Fair Oaks Lane, Frankfort, KY 40601 or call (502) 564-3410, or contact Third Rock Consultants, LLC, 2526 Regency Road, Suite 180, Lexington, KY 40503 or call (859) 977-2000. Hearing and speech-impaired persons can use the Kentucky Relay Service, a toll-free telecommunications device for the deaf (TDD). For voice to TDD, call 800-648-6057. For TDD to voice, call 800-648-6056.

Funding for this project was provided in part by a grant from the U.S. Environmental Protection Agency (USEPA) through the Kentucky Division of Water, Nonpoint Source Section, to Third Rock Consultants, LLC as authorized by the Clean Water Act Amendments of 1987, §319(h) Nonpoint Source Implementation Grant # M-05318649. Mention of trade names or commercial products, if any, does not constitute endorsement. This document was printed on recycled paper.

ACKNOWLEDGEMENTS

The following collaborators have had a strong, continuous involvement in the planning effort:

- Bill Payne, Lincoln County resident
- Boyle County Health Department
- Centre College Faculty and Staff
- City of Danville
- Clark's Run Environmental and Educational Corporation (CREEC)
- Garrard County Health Department
- Herrington Lake Conservation League
- Kentucky Division of Water
- Lincoln County Health Department
- Mercer County Health Department
- University of Kentucky - Kentucky Water Resources Research Institute
- USDA-NRCS, Boyle County office
- USDA-NRCS, Lincoln County office

EXECUTIVE SUMMARY

As part of the Kentucky Division of Water's (KDOW) 1998 Clean Water Action Plan, the Natural Resources Conservation Service (NRCS) and KDOW jointly selected five priority watersheds in Kentucky for targeted water quality improvement. The Dix River was selected as one of these priority watersheds.

Third Rock Consultants, LLC (Third Rock) was awarded a contract by the Kentucky Division of Water (KDOW) in 2006 to assist in the assessment of the Dix River Watershed. Third Rock collected samples or measurements on a total of 92 field days between March 2006 and July 2009. Samples were also collected from Herrington Lake, Mocks Branch and Spears Creek watersheds. Analysis of the Dix River Watershed was conducted in two phases: water quality monitoring and Microbial Source Tracking (MST).

As a result of the monitoring, six documents have been produced, namely the Dix River Watershed Monitoring Report, Clarks Run Watershed Based Plan, Hanging Fork Watershed Plan, a Nutrients TMDL for Clarks Run, and overviews of the Hanging Fork and Clarks Run watersheds.

Risks of disease due to human sewage and animal wastes were identified as the most serious impairment to the Dix River watershed. Additionally, poor aquatic habitat is common throughout the watershed, while specific areas are polluted by excessive nutrients that produce algal blooms that can reach levels toxic to fish and other aquatic life. Dissolved ions and the rapid changes in water levels due to storm runoff were also found to be significant problems.

Implementation will focus on specific best management practices (BMPs) to target the most significant of the identified impairments. To facilitate the remediation, several entities have been identified. The current establishment of the Stormwater Management Fund shows promise toward reducing nitrogen inputs as well as decreasing the velocity of stormwater entering Clarks Run. The strong involvement of local watershed and environmental groups such as CREEC, Healthy Planet Initiatives, Herrington Lake Conservation League, and KRWW show broad-based community interest and support of water quality improvements in the larger watershed. In addition, the relationship between Centre College and these groups provides a large volunteer base for watershed projects.

Table of Contents

Tables

Exhibit

Appendices

I. INTRODUCTION AND BACKGROUND

Herrington Lake, in the Kentucky River Basin, was formed by the impoundment of the Dix River. As is common with many reservoirs, Herrington Lake is subject to excessive nutrient loading resulting from point and nonpoint source contributions within the watershed. The Dix River Watershed contains failing septic systems, agricultural activities including numerous cattle with free access to streams, and development/construction activities. The resulting abundant nutrient input has led to the deterioration of water quality, problematic algal blooms, and subsequent fish kills.

Herrington Lake was listed in the 2004 303(d) report as a 1st priority impaired waterbody for aquatic life (non-support) and fish consumption (partial support). The major tributaries to the reservoir, Dix River, Clarks Run, and Hanging Fork, were also cited in the 2004 303(d) report as having segments listed as 1st priority impaired in regard to aquatic life support and primary contact (non-support and partial support). The cited reasons for impairment are primarily low levels of dissolved oxygen (DO) and high levels of bacteria. Sources of both impairments were believed to stem from agricultural runoff, septic tank leakage, urban/suburban stormwater runoff, and wastewater treatment plant discharges (USGS 2000).

As part of the Kentucky Division of Water's (KDOW) 1998 Clean Water Action Plan, the Natural Resources Conservation Service (NRCS) and KDOW jointly selected five priority watersheds in Kentucky for targeted water quality improvement. The Dix River was selected as one of these priority watersheds. Since that time, several groups have performed monitoring and analysis to provide date for remediation of the Dix River Watershed to a fully supporting status. In 2005, a monitoring analysis of Peyton Creek and Frog Branch, two tributaries of Hanging Fork, was conducted by Cumberland Environmental Group under the direction of the Heritage RC&D Council (2005). Results showed that Peyton Branch was more severely impacted than Frog Branch.

As a continuation of the effort to identify and eliminate pollutant sources, Third Rock Consultants, LLC (Third Rock) was awarded this grant by the Kentucky Division of Water (KDOW) in 2006 to assist in the assessment of the Dix River Watershed. Third Rock collected samples or measurements on a total of 92 field days between March 2006 and July 2009. Analysis of the Dix River Watershed was conducted in two phases: water quality monitoring and Microbial Source Tracking (MST).

The purpose of this data collection effort was to support a Total Maximum Daily Load (TMDL) development and watershed planning. A TMDL identifies pollutant sources and the amount of pollutants from each source, and makes recommendations for pollutant loads a stream can handle without violating water quality standards. The watershed plan is "a means to resolve and prevent water quality problems that result from both point source and nonpoint source problems" (USEPA 2005a).

II. MATERIALS AND METHODS

The total area of the Dix River Watershed includes approximately 282,000 acres in central Kentucky extending through Garrard, Mercer, Boyle, Lincoln, Casey, and Rockcastle counties. A total of 92 sampling sites were established within the watershed during the course of the project, 31 for the water quality monitoring study and 74 for the MST study, with 13 common sites monitored during both studies. Water quality monitoring sites were located in three main divisions of the Dix River Watershed: Hanging Fork, Clarks Run, and Upper Dix River watersheds. The lower portion of the Dix River, including Herrington Lake and its tributaries, was also monitored, but this data was not analyzed as part of this project. Exhibit 1, page 3, shows the locations of the monitoring sites.

The Clarks Run watershed covers about 18,000 acres or 28.5 square miles in Boyle and Lincoln counties, including about two-thirds of the City of Danville. Clarks Run and Balls Branch are the major streams draining the area. This watershed is in the inner subregion of the Bluegrass Physiographic Region, which is characterized by undulating terrain and moderate rates of both surface runoff and groundwater drainage. The watershed lies partly above fractured shales through which groundwater can easily move, but which store very little water. Other sections of the watershed lie over interbedded clay shales and siltstones. Land use in the area is approximately 70 percent agriculture, 12 percent residential development, 11 percent commercial/industrial, and 7 percent forest. Several factories are located adjacent to the creek and have associated stormwater discharge permits. As the stream exits the city limits, it flows past a limestone rock quarry and concrete plant. Eleven businesses hold permits to discharge in the watershed, including the City of Danville's wastewater treatment plant.

The Hanging Fork watershed covers about 61,000 acres or about 95 square miles in Boyle, Lincoln, and a small portion of Casey County, draining the cities of Junction City and Hustonville. Tributaries to Hanging Fork include Blue Lick Creek, Martin's Branch, Peyton Creek, Knoblick Creek, White Oak Creek, Harris Creek, Spears Creek, Baughman Branch, and Frog Branch. As the Hanging Fork watershed includes portions of both the Knobs and Outer Bluegrass ecoregions, the geology of the area is complex and includes both limestone and shale bedrock. Land use is primarily agricultural (64 percent) and forested (34 percent) with a small percentage of urban areas.

The upper Dix River watershed drains approximately 140,000 acres or 220 square miles (excluding Hanging Fork), in southern Garrard County, western Rockcastle County, and eastern Lincoln County. The land is characterized by undulating terrain, moderate to rapid surface runoff, and moderate rates of groundwater drainage. The watershed lies partly above fractured shales through which groundwater can easily move but which store very little water. The upper watershed of the Dix River includes the headwaters down to the Dix at KY 52 site, with the exception of Hanging Fork. The streams that feed it include Negro Creek, Turkey Creek, Copper Creek, Fall Lick, Drakes Creek, Harmons Lick, Walnut Flat Creek, Cedar Creek, Stingy Creek, Logan Creek, White Oak Creek, and Gilberts Creek. Land use in the Upper Dix watershed is 60 percent agricultural and almost 40 percent rural and forested. The cities of Stanford, Lancaster, Crab Orchard and Brodhead are located in this area.

N

Dix River, Herrington Lake

Clarks Run

Hanging Fork

Upper Dix River

Herrington Lake

Stanford Reservoir

Cedar Creek Lake

BOYLE

LINCOLN

CASEY

GARRARD

ROCKCASTLE

Danville

Junction City

Hustonville

Lancaster

Stanford

Crab Orchard

West Hustonville

Hanging Fork

Peyton Creek

McKinney Branch

Blue Lick Creek

Balls Branch West

Frog Branch

Baughman Branch

North Tributary

Hanging Fork Mainstem and Tributaries

Water Quality Monitoring Site

MST Sampling Site

Stream

City Boundary

Lake

Subwatershed Boundary

Watershed Boundary

County Boundary

Dix River

Mocks Branch

Clarks Run

Corporate Drive

Clarks Run at US 127 Bypass

South Second Street

Clarks Run at Goggins Lane

Clarks Run at KY52

Balls Branch Mouth

Clarks Run at Stanford Road

Balls Branch

Hanging Fork at Mouth

Dix at KY 52

Boone Creek

Turkey Creek

Dix Above Hanging Fork

White Oak Creek

White Oak Creek

Junction City

Knoblick Creek

Oak Creek

Knob Lick

Moores Lane

Hanging Fork Creek

Hanging Fork at US 150

Hawkins Branch

Gilberts Creek

Gilberts Creek

Logan Creek

Logan Creek

Stingy Creek

Drakes Creek

East Fork Drakes Creek

Harris Creek

McCormick Church

Blue Lick

Blue Lick Creek

Saint Asaph Creek

Walnut Flat Creek

Harmons Lick

Indian Branch

Fall Lick

Drakes Creek

Crab Orchard

Neals Creek

Spears Creek

Baughman Creek

Chicken Bristle

McKinney Branch

Peyton Creek

Martins Branch

McKinney Branch

Cedar Creek

Flax Creek

Copper Creek

Copper Creek

Gum Sulfur

Grassy Branch

Long Branch

Turkey Creek

2 1 0 2 Miles

Exhibit 1
Dix River Sampling Sites
Watershed Monitoring Report
Dix River Watershed

City, county, lake and watershed boundaries downloaded via the Kentucky GeoNet. National hydrography dataset downloaded from the Kentucky Office of GIS at <http://ogi.ky.gov/gisdata.htm>.

The field collection effort was divided into two phases, the initial water quality monitoring and a subsequent microbial source tracking survey. The quality assurance project plans which directed each of these data collection efforts are attached in Appendix B. The methods used in each of these data collection efforts is discussed below:

A. Water Quality Monitoring Summary

In the first phase, water quality monitoring, samples were collected and measurements recorded with the goal of determining the source and extent of impairment in the Dix River and its tributaries. Monitoring was performed during 70 field days from March 2006 to February 2007.

Before establishing sites, major reaches on Hanging Fork, Clarks Run, and the Upper Dix River were visually surveyed to optimize sampling station representativeness relative to nonpoint and point source contribution. Thirty-one sampling sites were established at representative locations to characterize the type and extent of impairments throughout the watershed. All 31 sites were sampled during low, normal, and high flows. Permanent monuments along with photographs and GPS points were established to standardize collection and measurement. At all sites, monthly grab samples were collected and analyzed for the parameters listed in Table 1. A description of each of these monitoring parameters and their effect on the stream or watershed may be found in the glossary. Microbac Laboratories, formerly Envirodata Group, analyzed total coliform and *E. coli* samples throughout the project, and chemical samples for March, April, and May 2006. CT Laboratories analyzed chemical parameters from June 2006 to February 2007.

EPA Rapid Bioassessment Protocol (RBP) worksheets were completed by Third Rock field technicians at all sites during the initial and final site visits. This assessment evaluated the multiple habitat parameters providing categorical as well as overall scores.

TABLE 1 – SAMPLING PARAMETERS FOR ALL MONITORING SITES

ONSITE	CHEMICAL	BACTERIOLOGICAL
Conductivity	Ammonia, un-ionized (NH3)	Coliform, Total
Depth	Carbon, Total Organic (TOC)	*Escherichia coli* (E. coli)
Discharge	Nitrate-Nitrogen (NO3)	
Dissolved Oxygen (DO)	Nitrite-Nitrogen (NO2)	
pH	Nitrogen, Total Kjeldahl (TKN)	
Temperature	Orthophosphate (OP)	
	Phosphorus, Total (P)	
	Solids, Total, Suspended (TSS)	

At 11 select sites, additional monitoring was performed for the parameters and frequency specified in Table 2. Also, continuously monitoring pressure transducers were established at six sites throughout the Dix River watershed to record the fluctuations in the stream water levels every 20 minutes for the duration of the project.

TABLE 2 – SELECT SITE SAMPLING PARAMETERS

PARAMETER	FREQUENCY
Alkalinity, Total as CaCO3	Monthly
BOD, 5-Day	Monthly
BOD, 15-Day	Monthly, (Clarks Run select sites only)
Chlorophyll a	Monthly
Turbidity	Monthly
Chloride	Quarterly
Periphyton	June 5-6 and September 5-6, 2006
High flow stormwater event for all chemical parameters	January 5, 2007

Because of known impairments in the watershed areas, several sites were also monitored further in the Clarks Run and Hanging Fork watersheds. On January 5, 2007, a storm flow sample was collected at all select sites. Because of the suspected dissolved oxygen impairment in Clarks Run, monthly samples were collected and analyzed for 5-day biochemical oxygen demand (BOD5) at all sites and for 15-day BOD at select sites in this watershed. Also, 24-hour diurnal dissolved oxygen measurements were taken at two stations in Clarks Run on August 16, 2006 and at two different locations on July 31, 2008 and August 6, 2008. Because pathogens were a known concern in the Hanging Fork watershed, samples were collected while the streams were rising due to a storm event on September 18, 2006 at all sites in this watershed.

Because during the original sampling the laboratory instrument sensitivity for phosphorus and nitrogen data did not provide the resolution necessary to meet the data quality objectives for TMDL development, an additional eight sampling events for nutrients, physiochemical parameters, total organic carbon, biochemical oxygen demand, and discharge were collected at each of the original eight sites in Clarks Run from December 2008 to July 2009.

Specific methods utilized in these collection efforts are detailed further below:

1. Sampling Station Locations and Specifications

The specific criteria for site location are discussed below. Due to logistical constraints, stations were commonly located in proximity to bridge crossings or culverts. Care was taken when locating stations so that sampling sites were far enough away from bridges or culverts to minimize the influence of the inherent hydrologic modification caused by anthropogenic modifications.

Sites in the Clarks Run subwatershed were located to discern nutrient and bacterial contributions from non-point sources (primarily cattle and residential), industrial facilities, potential sewage collection failures, and point-source contributions. The Hanging Fork watershed is characterized primarily by agriculture (pasture) with a scattering of small communities having sanitary sewer outfalls, so stations were positioned to help pinpoint the location of major sources of nutrient and bacteria contribution from this watershed. The Upper Dix River area, similar to the Hanging Fork subwatershed, contains primarily

agricultural pasture with rural residences and small communities (with WWTP outfalls). Though the data from these sites was not specifically to be used for TMDL calculation, the resultant information will help determine and rank the significance of nutrient, TSS, and bacteria contribution of this drainage to Herrington Lake.

During all sampling activities, sampling methods and gear utilized was analogous to USEPA and KDOW recommendations. Specific methods are detailed in the following sections. All samples were collected in bottles according to the analytical methods referenced in Table 3, Summary of Project Sampling and Analytical Requirements, shown on page 6.

Samples were collected directly from the source. When collecting samples, latex gloves were used to prevent contamination. The sampling technician collected the sample by submersing a decontaminated rinsed stainless-steel bucket into source as to obtain a representative aliquot. Submersion was only to the bucket mid-depth, taking caution not to scrape the bottom of the source to minimize excess solids. An appropriately sized bucket relative to the bottle(s) being collected was used. The bucket size was sufficient to completely fill the sample bottle(s) from a single submersion, taking care to avoid overfilling in bottles containing preservative. Pre-labeled collection bottles were filled per method specifications directly from the bucket.

Stream samples were collected from the thalweg (or low water channel) just above the stream bottom. Bottles were filled to near 100 percent capacity. Efforts were made not to stir up sediments during collection. Proper field data sheets were completed. Samples were labeled accordingly, placed on ice, and delivered to CT Laboratories Laboratory within the required holding time(s). Proper chain-of-custody procedures were followed to ensure accuracy in sample reporting. Field quality controls were also collected at this time. Care was taken when filling total organic carbon (TOC) sample bottles to avoid unnecessary agitation of water and to ensure complete filling of bottle, as headspace in the bottle could cause bias of results due to volatilization of organic carbon.

TABLE 3 – SUMMARY OF PROJECT SAMPLING AND ANALYTICAL REQUIREMENTS

Parameters	Analyte Name	Method	Minimum Sample Volume	Containers	Preservation	Maximum Hold Time
CBOD15	Biochemicial Oxygen Demand, 15-Day Carbonaceous	EPA 405.1 MOD or SM5210B MOD	1 L	Plastic	Cool 4°C	48 hrs
CBOD5	Biochemicial Oxygen Demand, 5-Day Carbonaceous	EPA 405.1 MOD or SM5210B MOD	1 L	Plastic	Cool 4°C	48 hrs
TSS	Solids, Total Suspended	EPA 160.2	1 L	Plastic	Cool 4°C	7 days
Total P	Phosphorus, Total	EPA 365.1 or 365.4	50mL	Plastic	Cool 4°C, H_2SO_4 to pH <2	28 days
Ortho-P	Phosphorus, Ortho	EPA 300.0 or 365.2	250mL	Plastic	Cool 4°C	48 hrs
NO2	Nitrite as N	EPA 300.0	50ml	Plastic	Cool 4°C	48 hrs*
NO3	Nitrate as N	EPA 300.0	50mL	Plastic	Cool 4°C	48 hrs*
NH3-N	Ammonia as N	EPA 350.1	500mL	Plastic	Cool 4°C, H_2SO_4 to pH <2	28 days
Chloride	Chloride	EPA 300.0	25mL	Plastic	Cool 4°C	28 days
TKN	Total Kjeldahl Nitrogen	EPA 351.2	50mL	Plastic	Cool 4°C, H_2SO_4 to pH <2	28 days
TOC	Organic Carbon, Total	EPA 415.1	25mL	Amber Glass	Cool 4°C, H_2SO_4 to pH <2	28 days
Alkalinity	Alkalinity	EPA 310.1 or 310.2	100mL	Plastic	Cool 4°C	14 days
Turbidity	Turbidity	EPA 180.1	Sufficient volume to submerge probe	Direct source measurement	NA	On-Site [1]
pH	pH	EPA 150.1			NA	Immediately/On-Site
DO	Dissolved Oxygen	EPA 360.1			NA	Immediately/On-Site
Temp	Temperature	EPA 170.1			NA	Immediately/On-Site
Cond	Conductivity	EPA 120.1			NA	On-Site [1]
Flow	Flow	USGS Modified	NA	NA	NA	NA
TC/EColi	Total Coliform / E. coli	SM 9223	100mL	Glass/Plastic, Sterile	Cool <10°C, $Na_2S_2O_3$ (No Cl_2)	24 hrs
Chloro a	Chlorophyll a	SM 10200H**	Varies	Amber Glass	***	****
Periphyton	Periphyton	Douglas, 1958	Varies	Amber Glass	See Note[2]	NA
24hr. Diurnal DO	24hr. Diurnal Dissolved Oxygen	EPA 360.1	Sufficient volume to submerge probe	Direct source measurement	NA	Immediately/On-Site

* Optional preservation of 250 mL with H2SO4 (1+1) to a pH <2 results in a holdtime of 28 days for Nitrate-Nitrite.

** Trichromatic

*** Cool, 4°C, Protect From Light - Wrap Amber Glass Bottle in Aluminum Foil

**** Concentrate sample as soon as possible after collection. *Filter* samples from waters w/ pH =/> 7.0 can be placed in air tight bag

and stored frozen for 3 weeks; *filter* samples from waters w/ pH <7.0 should be processed as soon as possible to prevent chlorophyll degradation.

[1] Samples can be collected for laboratory analysis: Turbidity - 100mls, plastic, cool 4^{o}C, 48hr hold; Conductivity - 100mls, plastic, cool 4^{o}C, 24hr hold if sample is unfiltered/28 day hold if sample is filtered through 0.45um membrane filter.

[2] Lugol's iodine solution, 0.3mL per 100mL of sample

During initial setup of the site locations, several tasks were completed at each station:

- Permanent monuments (survey pins) were established to standardize water collection, flow measurement, and photograph locations at each station.
- Passive high flow storm-water sampling device locations were determined and installed (select stations only).
- Cross-sectional measurements were completed at each station to support discharge computation. For each cross-section, three reference points were established. Two of the points, located on opposite sides of the bank, were located for subsequent section measurements. The third point was located for reference of stage (tape-down) readings. Stage reference points may be located on a bridge, established with pins (rebar), or a sturdy overhanging limb. This work was done to aid in the measurements as listed below:

2. Habitat

During habitat assessment, at the initial and final station visits, a thirty-minute visual inspection was completed at each stream sampling station or reach. Ten habitat parameters were assessed, according to Methods of Assessing Biological Integrity of Surface Waters in Kentucky (KDOW 2002). Each parameter was rated (1 to 20) and combined to obtain a habitat score (0 to 200) that was compared to a reference condition. Use attainment was estimated based on the habitat score.

3. Flow

In order to determine stream discharge or flow (Q), the flow area (A) and water velocity (V) was measured. Flow was subsequently calculated according to the following equation for increments across the stream.

Flow Equation: $Q = V * A$

where: Q = Discharge or Flow (ft^3/sec)
V = Velocity (ft/sec)
A = Flow Area (ft^2)

In order to measure the flow area, three methods were used. For all stations, a stream cross section was surveyed (via Total Station). For six select stations, this information was used in conjunction with a pressure transducer water level recorder (Infinities USA) to determine the flow area. When the water level was measured at the cross-section with a staff gauge or marked with pins on the stream bank, the flow area was calculated. Alternatively, the stream was waded at the cross-section to determine depth, breadth, and velocity at the time of the sampling visit.

The flow for all wadeable streams was measured on a monthly basis according to USGS 2000. Velocity and water depth were measured at intervals across the stream sufficient to characterize discharge. A 100-ft tape was stretched across the stream in the established cross-section to indicate the intervals. Typically, stream depth and velocity were measured at 3 ft intervals across the stream. The interval was adjusted as necessary to thoroughly characterize the entire cross-section of flow. Points were closer together if there was significant variation in the depth or velocity of the cross-section.

To increase the accuracy of flow calculation, the first and last velocity and depth measurements were made as close to the banks as feasible. At each station within the cross-section, velocity was measured with a General Oceanic current meter mounted on a rod, where velocity was indicated by the number of revolutions of the propeller over a given time interval. The individual using the velocity meter held the rod vertically in the profile with the meter parallel to the direction of stream flow and stood at least 1 ft downstream and to the side of the velocity meter to avoid interfering with the current. Later, this method was replaced by a Marsh-McBirney Doppler velocity meter for greater accuracy in the typically shallow bedrock streams.

Average velocity was measured at 0.6 of total stream depth when the depth was less than 2.5 ft. When the stream was deeper than 2.5 ft, velocity was measured at 0.2 and 0.8 of the total depth and the average of the two readings was used as the average velocity at that point for discharge calculations. Discharge was calculated for each interval of the stream where velocity and depth was measured and total stream discharge was calculated as the summation of the discharge from each interval. Water depth was also recorded at a single known point in the stream during each visit.

When the stream was too deep to wade with the current meter, stream velocity was roughly estimated using a floating object. The float could be any buoyant object, such a partially filled plastic water bottle. Ideally, the object was heavy enough so that about an inch of it was below the water line. When the floating object could not be retrieved from the stream, a "weighty" yet compact piece of stick/wood was used. When feasible, a 50 ft section of stream was measured for the float test. The float was released out into the stream in a location most representative of the entire stream, and the time was recorded for it to travel the known distance. If the float moved too fast for accurate measurement, a longer travel distance was measured. The simple float estimation of velocity was repeated for a total of three trials. The surface velocity values obtained by this method were corrected to represent mid-depth velocity (Daugherty *et al*. 1985).

Discharge during high flow was estimated using this velocity measurement, cross-section information, and depth measured from the pressure transducer water level recorder, staff gauge, or pins on the bank.

4. Physio-Chemical Measurements

Temperature, dissolved oxygen, conductivity, and pH were measured during field sampling of the streams with a Hydrolab water quality instrument. Operation of the Hydrolab instrument was conducted in conformance to the Hydrolab operation manual (Hydrolab, 1997).

During the low-flow summer period, 24-hour diurnal dissolved oxygen was measured with the Hydrolab during three separate occasions at two sites on Clarks Run (two of which were below the WWTP). The Hydrolab was deployed for 24-hours during each event during which its data-logging feature stored the dissolved oxygen data.

Global Positioning System coordinates were obtained using a Garmin GPS accurate to ±5-40m. Readings were measured in NAD83. Internal SOPs and manufacturer's instructions were followed to record these measurements.

5. Periphyton Sampling

Periphyton sampling was conducted in accordance with the *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (KDOW 2002). To meet these objectives, the Sampling Logistics Coordinator built a Periphyton Substrate Vacuum. Based on KDOW 2002 methods, this vacuum consisted of a 3-inch diameter PVC pipe used in conjunction with a neoprene rubber gasket attached to a hand operated pump. To sample periphyton from stations, the gasket end of the PVC was pressed against the bedrock substrate so that the periphyton within the area enclosed was dislodged with a stiff bristle brush. The hand operated pump was then inserted into the PVC pipe (still being pressed against the bedrock) and the periphyton was pumped into a filer flask using the hand operated pump. Five replicates were taken for a total area of 0.25m^2. This portion was sent to the laboratory for analysis by a modified version of Douglas 1958.

6. Chlorophyll *a*

Chlorophyll *a* samples were filtered in Third Rock's lab before transporting to CT Laboratories for analysis. Initially, the time, date, and volume of the sample were recorded on a Third Rock bench sheet. A measured volume of water from each sample was filtered through 0.45µm cellulose membrane filters. For each sample, water was filtered and particulate matter was collected on three membrane filters, folded in half and enclosed within aluminum foil. Each sample was then placed in a zip-lock bag, labeled with the filtered volume of water, and frozen before delivery to the lab. The bench sheet accompanied the filtered sample with the information regarding date/time of collection, date/time of filtration, volume of filtered sample and area of aspiration.

7. High Flow Sampling

Sampling periods included an elevated storm flow between November and April with the goal of capturing one high flow per month during that period with a seven-day antecedent dry period. Teams of sampling technicians were deployed during potential collection periods and samples were collected during the rising stage of increased flow.

8. Pressure Transducer Water Level Recorder

At 6 of the 11 select locations, stream water level was continuously monitored using a pressure water level recorder (Infinities, USA). These sites included Drakes Creek, Dix Above, Knob Lick, Hanging Fork 150, Clarks Run Bypass, and Balls Branch Mouth. The pressure sensor measured water depth and digitally recorded the data on a user-defined interval. For this project, the device recorded water level readings every 20 minutes. The

pressure sensor was accurate to +/- 0.1 percent of the measurement range and the resolution was 0.01 inches.

9. Sampling Equipment

For the purposes of this project, the following equipment was utilized in the sampling effort:

- Periphyton Substrate Vacuum
- Filtration Apparatus
- Hydrolab MS5 and associated probes
- Infinities USA continuous pressure transducer water level recorder
- General Oceanic current meter
- Marsh-McBirney electromagnetic current meter
- Garmin GPS
- Turbidimeter

10. Quality Control

Chemical data quality was ensured through strict adherence to KDOW standards (2002b, 1995). Approximately 10 percent of water samples were duplicated or split and sent to CT Laboratories for analysis.

- Field Duplicate Sample

Approximately five percent of all samples taken in the field were duplicated. To perform a field duplicate, the Sampling Technician consecutively collected two representative aliquots, independent of one another, from the same source by the grab collection technique.

- Field Split Sample

Approximately five percent of all samples taken in the field were split. To perform a field split sample, the Sampling Technician evenly divided the contents of one grab collection into two sets of sampling bottles. To ensure that the split was representative, sample bottles were each filled in three rounds of filling each bottle one third of the total volume.

To ensure that data of known and documented quality are generated in the laboratory, the QC criteria described in this section were met for all analyses, as applicable. The Laboratory QA Director was responsible for monitoring and documenting procedure performance, including the analysis of control samples, blanks, matrix spikes, and duplicates.

- Blanks

A method blank (MB) was prepared at a frequency of one per 20 field samples depending on the specific method. The MB was analyzed at the beginning of every analytical run and prior to the analysis of any samples. MB results were acceptable if the concentrations of the target analyte did not exceed the reporting limit (RL). If any target analyte concentration in the MB exceeded the RL, the source of contamination was identified and eliminated. Analysis of samples did not proceed until a compliant MB was obtained.

- Duplicates

A duplicate sample (DUP) or duplicate matrix spike sample (MSD) was prepared at a frequency of one per 20 field samples depending on the specific method. The relative percent

difference (RPD) between duplicate samples, for samples having analyte concentrations greater than their respective reporting limit, or between a matrix spike (MS) and matrix spike duplicate (MSD), had to be within the acceptance ranges. If the QC criteria for duplicate sample or spike analyses were not satisfied, the cause of the problem was determined and corrected. If the problem adversely affected the entire analysis batch, all samples in the batch were reanalyzed.

- Matrix Spikes

Spikes (MS) were prepared every 20 field samples for each matrix, depending on the specific method. Spike recoveries had to fall within the acceptance ranges. If the QC criteria for the matrix spike analyses were not satisfied, the cause of the problem was determined and corrected. If the problem adversely affected the entire analysis batch, all samples in the batch were reanalyzed.

- Laboratory Control Samples

A laboratory control sample (LCS) was second-source to the calibration standards and was prepared at a frequency of one per every 20 field samples depending on the specific method requirements. The LCS results were acceptable if the percent recovery of each analyte was within the determined acceptance.

B. Microbial Source Tracking Summary

Due to the excessive total coliform and *E. coli* values observed from initial monitoring, the second phase of the project, Microbial Source Tracking (MST), focused on identifying and quantifying the sources of pathogen pollution in the Hanging Fork and Clarks Run subwatersheds. This analysis occurred in four steps:

1. Development of a GIS dataset of human wastewater sources
2. Site identification and characterization
3. *E. coli* and total coliform analysis for hotspot identification
4. Microbial Source Tracking of host sources

A GIS data set was developed for human wastewater sources for Boyle and Lincoln counties. This data set includes the type and age of wastewater treatment system, type of facility, location, and any general notes on the condition of the system. It was created based on three sets of information: local knowledge from County Health Department staff, property boundaries from the local Property Valuation Administrators (PVA), and sewer system coverage from the Kentucky Water Resource Information System (KY WRIS). If a property did not have sewer access, it was assumed to have a septic system. Health Department staff indicated known problem areas with failed septic systems and the age of the systems where known.

Owner information was provided with PVA data. For locations where both age and owner were unknown, no information was recorded. The results of this analysis were submitted as a GIS file to the KDOW.

Aerial photographs were surveyed for a combination of variables, including land use, site access, existing data implications, and potential source contributions, to establish potential sites for characterization and analysis. Using these variables, 74 sampling sites were established, characterized for visual signs of fecal inputs, and assessed for habitat during 10 field days in July 2007.

Because of drought conditions in 2007, sampling of these sites was delayed until May of 2008. *E. coli* and total coliform samples were collected and analyzed by Third Rock at 72 sites during a storm event May 9 and a normal flow event May 27. *E. coli* was utilized to indicate the pathogen loading of the watershed and the atypical to typical coliform colony ratio analysis (AC/TC) associated with the total coliform to indicate the fecal age and the general source.

Analysis of these results indicated 20 "hotspots" for further analysis. Hotspot sites were chosen based on representativeness of overall watershed area, the number of upstream sites, *E. coli* concentration, number of suspected sources to be resolved, and land use. A normal flow event on June 22 and a storm flow event on July 4, 2008 were sampled for analysis at Source Molecular Laboratories for the following methods:

- Human Enterococcus ID
- Human Bacteroidetes ID
- Cow Enterococcus ID
- Cow Bacteroidetes ID

All samples that tested positive for any of these parameters were further analyzed by quantitative polymerase chain reaction (qPCR) methodology to quantify the relative contribution of each host source to the total. The quantitative contributions were produced based on comparisons to samples collected from the Danville wastewater treatment plant and a commercial stockyard.

III. RESULTS AND DISCUSSION

A. Monitoring Results

The total area of the Dix River Watershed includes approximately 282,000 acres in central Kentucky extending through Garrard, Mercer, Boyle, Lincoln, Casey, and Rockcastle counties. A total of 92 sampling sites were established within the watershed during the course of the project, 31 for the water quality monitoring study and 74 for the MST study, with 13 common sites monitored during both studies. Water quality monitoring sites were located in three main divisions of the Dix River Watershed: Hanging Fork, Clarks Run, and Upper Dix River watersheds. The lower portion of the Dix River, including Herrington Lake and its tributaries, was also monitored but the results are not included in the scope of this monitoring report.

All water quality monitoring sites are labeled according to area landmarks. Because of the high number of MST sites, sites were identified by an alphanumeric system, with the letters signifying the watershed division in which they were located.

Discharge was evaluated at all sites, and at some sites was compared to weather patterns to provide an indication of the amount of fluctuation in the stream levels, the water volume associated with the sample collection effort, and the relationship to precipitation.

In order to evaluate the nature and extent of impairments in the Dix River Watershed, results were compared to applicable water quality benchmarks. The benchmarks used in this comparison were of multiple types, including legal limits as well as scientific evaluations.

For parameters are listed in 401 KAR 10:031, the legally binding surface water standards for warm water aquatic habitat in Kentucky were used as the benchmark. Specific criteria are listed for dissolved oxygen, pH, water temperature, chloride, un-ionized ammonia, fecal coliform, and *E. coli* as shown in Table 4 (page 14). Water quality standards for metals and pesticides/herbicides are also available, but have not been listed herein due to the infrequency in the data collection of these parameters in this watershed. For specific conductance, flow, total suspended solids, and alkalinity, specific standards are not provided, but 401 KAR 10:031 indicates than levels "shall not be changed to the extent that the indigenous aquatic community is adversely affected." Nutrients in surface waters are also to be regulated such that "where eutrophication problems may exist, nitrogen, phosphorus, carbon, and contributing trace element discharges shall be limited in accordance with: (1) the scope of the problem; (2) the geography of the affected area; and (3) relative contributions from existing and proposed sources."

For total phosphorus and total nitrogen, the Kentucky Division of Water has specified a numeric target for Clarks Run in association with the development of total maximum daily loads (TMDL). The TMDL is the maximum amount of a pollutant that a waterbody can receive and still safely meet water quality standards, thus the target is used as the benchmark for these parameters. The TMDL target for total phosphorus is 0.3 mg/L and for total nitrogen the target is 2.0 mg/L.

TABLE 4 – KENTUCKY SURFACE WATER STANDARDS

PARAMETER	UNIT	KY WQS		ADDITIONAL COMMENTS
		CHRONIC	ACUTE	
Dissolved Oxygen	mg/L	5	4	5.0mg/L is minimum daily average; 4.0 mg/L is instantaneous minimum
pH	SU	6.0/9.0		pH shall not fluctuate more than 1.0 SU over a period of 24 hours.
Temperature	deg. F		89	
Chloride	mg/L	600	1200	
Ammonia, un-ionized	mg/L		0.05	Un-ionized ammonia is determined based upon the pH, temperature, and total ammonia-N concentrations.
Fecal Coliform	cfu/100mls	200	400	There are not chronic and acute criteria for bacteria, but a geometric mean for five samples collected over 30-days and instantaneous criteria, respectively.
E. coli	cfu/ 100mls	130	240	

Where no specific legal standard was present, benchmarks are provided for comparison purposes and have no regulatory / legal force. The US EPA Storage and Retrieval (STORET) database was used to provide comparisons based on 39576 results for the state of Kentucky and 18229 results from the Interior Plateau ecoregion of Kentucky collected between 1990 and 1997 (USEPA 2009a). For parameters for which data was sufficient data was available, Table 5 (page 15) summarizes the number of sample results available, the arithmetic average, and the 25^{th}, 50^{th}, 75^{th}, and 95^{th} percentiles. Percentiles indicate the value at which that percentage of the results is below when all the results are ranked from lowest to highest (for example, 25% of the results are below the 25^{th} percentile). These results were used to evaluate whether results are low, moderate, or high.

TABLE 5 – USEPA STORET DATABASE BENCHMARKS

PARAMETER	UNIT	INTERIOR PLATEAU						STATEWIDE					
		# SAMPLES	MEAN	PERCENTILE				# SAMPLES	MEAN	PERCENTILE			
				25TH	50TH	75TH	95TH			25TH	50TH	75TH	95TH
Ammonia Nitrogen, Total	mg/L	3052	0.06	0.02	0.02	0.05	0.195	5877	0.06	0.01	0.01	0.05	0.2
Nitrite and Nitrate	mg/L	3049	1.02	0.27	0.69	1.28	3.34	5893	0.75	0.19	0.44	0.93	2.61
Total Kjeldhal Nitrogen	mg/L	2635	0.52	0.24	0.42	0.645	1.34	5223	0.44	0.16	0.32	0.57	1.21
Phosphorus, Total	mg/L	2832	0.16	0.03	0.08	0.19	0.63	5707	0.11	0.01	0.03	0.11	0.45
Total Suspended Solids	mg/L	131	75.6	16.5	35	76	357	174	70.6	12.3	32	72	355.5
Turbidity	NTU	1732	32.1	10	21	37.3	120	4998	12.0	0.05	0.59	9	69
Conductivity	µS/cm	See Note						7044			295		771
Alkalinity, Total	mg/L							4334			100		202
Carbon, Total Organic	mg/L							4338			2.37		6.76
Sulfate	mg/L							4345			34		271

Note: Interior Plateau data not available for these parameters. Statewide values based on KDOW collected STORET data in USEPA 2006.

In cases where no STORET data was available, other applicable benchmarks were used to evaluate the water quality. The common KPDES permit of 10 mg/L was used to evaluate BOD levels. The conductivity level of 500 µS/cm is used as a benchmark considering levels above this limit may not be suitable for macroinvertebrates and fish (USEPA 2009b).

Habitat values are evaluated according to the standards found in KDOW's *Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (2008). Each habitat parameter is evaluated as "optimal," "suboptimal," "marginal," or "poor," and the total of these scores is evaluated as "fully supporting," "partially supporting," or "not supporting" according to the Bluegrass bioregion standards and the upstream watershed size, as shown in Table 6.

TABLE 6 – HABITAT CRITERIA FOR BLUEGRASS BIOREGION STREAMS

RATING LEVEL	WADEABLE STREAM (>5 MI^2 WATERSHED)	HEADWATER STREAM (<5 MI^2 WATERSHED)
Fully Supporting	130 and above	156 and above
Partially Supporting	114 – 129	142 – 155
Not Supporting	113 and below	141 and below

In the MST study, laboratory testing was conducted to compare the ratio of atypical coliform colonies (AC) to typical coliform colonies (TC), or the AC/TC ratio, found in water samples to indicate the freshness of the fecal input. This ratio can be used to broadly indicate the source of the fecal input as shown in Table 7. According to research, AC/TC ratios of less

than 2 indicate extremely fresh sources, often attributable to human input. Direct agricultural inputs tend to be somewhat higher, in the range of 2 to 4, but ratios below 2 for this source can occur. More aged sources have a higher ratio. Ratios below 2 were the most abundant throughout the watershed.

TABLE 7 – RELATIONSHIP OF AC/TC RATIO AND SOURCE CONTRIBUTION

AC/TC RATIO	DESCRIPTION
< 2	Fresh, likely human source
2 - 4	Fresh, human or agriculture sources
4 -10	Moderate age, likely indirect agriculture
10- 20	Older, likely indirect urban
>20	Aged, human or agriculture sources

For the DNA testing conducted, two bacterial taxa, *Bacteroidetes* and *Entercoccus* sp., were utilized in order to indicate the source of the input. *Bacteroidetes* and *Entercoccus* are bacteria normally present in the gastrointestinal systems of humans and warm-blooded animals but occur in stream environments only by fecal deposition. The testing methods are designed around the principle that certain strains of these bacteria are specific to humans or cattle, and DNA markers can be used to detect these strains. Because these specific markers are not present in every strain that inhabits humans or cattle, a human or cattle input present may not always be detected, but a positive result predicts presence of the source with almost perfect reliability. The percentages of fecal inputs attributed to human and cattle sources in this study are approximations based upon comparison of the stream results with results from samples collected from human sewage and cattle fecal reference samples. These percentages have an accuracy of +/- 20 percent. When percentages do not total 100 percent, it should not be assumed that fecal contributions are due to wildlife or some other source, but that the source is currently unknown at this time.

Monitoring results are summarized by subwatershed and are discussed below.

1. Clark's Run

As a result of the monitoring study, additional streams in the Clarks Run watershed have been identified as impaired and listed on the 2008 303(d) list of impaired surface waters. Risks of disease due to human sewage and animal wastes have been identified as the most serious impairment to the watershed. Poor aquatic habitat is common throughout the watershed, while specific areas are polluted by excessive nutrients, which produce algal blooms and reaches levels toxic to fish and other aquatic life in certain areas. Dissolved ions and the rapid changes in water levels due to storm runoff are also significant problems in Clarks Run. Each of these impacts identified in the monitoring study are explained in more detail below.

Algal Blooms and Absent Riparian Zone on Sampled Stream

- Habitat

Habitat assessment evaluates the physical structure of streams to determine how these factors influence water quality. Healthy streams provide diverse habitat for numerous species, and the assessment also determines the potential for the stream to provide habitat. The Kentucky Division of Water has established three categories to rank the quality of the habitat a stream provides, listed in the order of best to worst: "fully supporting," "partially supporting," and "not supporting."

Of the twenty-two sites surveyed in Clarks Run, the majority of the sites were determined to have poor habitat, with over 75 percent either "not supporting" or only "partially supporting" habitat use. Although the reasons for the poor habitat designations differed from site to site, common trends were observed in the watershed.

Habitat was most commonly reduced throughout the watershed because the vegetated area adjacent to the stream, called the riparian zone, was either absent or underdeveloped. The riparian zone is important because it provides wildlife habitat, reduces stream erosion, filters nutrients, traps sediment, and provides canopy cover to the stream. Improving the riparian area by vegetating the area within 60 feet of each stream bank with native plant species and reducing disturbance (human activity, livestock damage, etc.) will provide the greatest improvement to stream habitat.

In the agricultural areas of the watershed, such as Balls Branch West, some of the poorest habitats were frequently due to impacts from cattle grazing along the creek and trampling the banks, creating erosion that impacts aquatic habitats with sediment. In urban areas, the rapid delivery of runoff to streams during storms was also causing erosion and the subsequent deposition of sediment into insect and fish habitats, although these impacts were usually less severe than those in agricultural areas.

In general, the habitat of streams and tributaries near the outer boundaries of the watershed was much more impacted than on the larger Clarks Run. Although the habitat of all streams in the watershed was not assessed, these trends appear representative of streams throughout the watershed.

At one site, Clarks Run at Goggin Lane, frequent dumping of garbage and other litter was observed and should be addressed.

- Pathogens

Pathogens are organisms that are capable of causing disease, such as bacteria, viruses, protozoa, or fungi. Pathogens enter streams through sewage or animal wastes and present a health risk to people who use the streams recreationally. Because testing for individual pathogens is unfeasible, *E. coli* was sampled as an indicator of sewage or animal wastes in streams within the Clarks Run watershed. Results indicate that concentrations of *E. coli* often ranged from ten to one hundred times greater than the statewide limit. Thus, the most significant impact in Clarks Run is the fecal pollution of the watershed.

Because the concentrations of *E. coli* were relatively high, additional testing was conducted to identify the source of the fecal inputs and the relative concentrations of individual tributaries. Balls Branch West showed the highest concentrations of *E. coli* in the watershed; therefore, additional sampling sites focused on the upstream tributaries in this area. Other sampling sites were established along the main stem of Clarks Run and its tributaries, although the highest average concentrations in this area occurred at the crossing of Stanford Road.

In the Balls Branch subwatershed, the most concentrated input was traced to the neighborhoods clustered around US 127. Seventy percent of the contribution was indicated as human by DNA testing, and 15 percent was due to cattle. Much of this human contribution is suspected to have originated from overflows at the upstream sewage pump station, currently being upgraded by the City of Danville. On the southern tributary to Balls Branch along Goose Pike, DNA tests indicate that cattle contributions were more significant at 50 percent, while human sources composed only 10 percent. The remaining percentage may be due to human, cattle, or other sources, but is currently unknown. It is expected that the western tributary to Balls Branch is similarly more influenced by cattle fecal sources. Thus, both human and cattle inputs were impacting Balls Branch, but human sources caused the most concentrated inputs.

Along Clarks Run, DNA testing was conducted at two sites to identify fecal sources. At the Stanford Road crossing, 80 to 100 percent of the contribution was identified as human, while on a tributary to Clarks Run between South Second Street and the US 127 Bypass equal contributions were identified from human and cattle sources. These results indicate that sewage systems, whether sewer or septic systems, are the source of the most concentrated fecal contributions, and cattle sources contribute to a lesser degree.

- Nutrients and Algal Blooms

Although nutrient levels are somewhat elevated throughout the Clarks Run watershed, concentrations of phosphorus and nitrogen compounds are well above acceptable levels at the two sites downstream from Danville's wastewater treatment plant (WWTP), which is located between Stanford Road and KY 52.

Concentrations of organic nitrogen and nitrate were approximately three times higher than those measured at most other locations in the watershed on average downstream of the WWTP near the overpass of KY 52 and also near Goggin Lane. In addition, un-ionized ammonia (a form of nitrogen) was also significantly elevated below the WWTP. Although no regulatory limits have been established for nitrate or organic nitrogen (TKN), concentrations were routinely above the average for the ecoregion. The elevated concentrations of these forms of nitrogen are created by the decomposition of human waste, animal manure, cleaning products, or fertilizer. For un-ionized ammonia, concentrations below the WWTP were found to exceed the regulatory standard. Levels above that standard have been found to impact fish and macroinvertebrate species and even cause death to aquatic organisms.

Phosphorus levels downstream of the WWTP were similarly two to three times higher than the concentrations at sites not influenced by the treatment plant. Sources of phosphorus include fertilizer, detergents, decomposition of plants or food, and human or animal waste.

Algal Bloom at Goggin Lane

Algal blooms are rapid increases in a stream's algal population and are caused by an abundance of nitrogen, phosphorus, and sunlight. Algal blooms were observed throughout the watershed, but were especially dense near Goggin Lane, where they were clogging the entire stream. Algal blooms also occurred at the overpass of KY 52, but shading of the stream by tree canopy minimized the severity of these blooms.

Algal blooms impact streams in a number of ways. The unattractive appearance can detract from the recreational value of the stream, causing property values to decline. Because of their volume, they also reduce habitat for some aquatic species. Although the two sites monitored for nighttime dissolved oxygen levels were normal, algal blooms are known to cause fish kills by reducing nighttime dissolved oxygen concentration to toxic levels. No dissolved oxygen problems were detected in Clarks Run, most likely due to frequent aeration at riffles in the shallow streams.

- Dissolved Ions

Conductivity is a measurement of the stream's ability to carry an electrical current. In streams, this is dependent on the concentration of inorganic dissolved solid ions such as nutrients, metals, or other compounds in the water. All sites had conductivity levels averaging above levels in which sensitive aquatic insects, such mayflies, are impacted. Clarks Run at Goggin Lane, KY 52, Stanford Road, and South Second Street each averaged levels that have been shown to impact fish species. The excessive nutrient concentrations, along with natural ions and other pollutants, contribute to these high conductivity values impairing the stream.

- Stream Flashiness

Although not specifically investigated as part of this study, stream gauging stations indicate that the streams of Clarks Run are "flashy," with large volumes of water rapidly flowing into and out of the stream system during storm events. In normal stream environments, most rainfall is absorbed into the ground, which filters out many nutrients and other pollutants as rainfall slowly passes into the stream. However, in areas with high percentages of impervious surfaces and efficient stormwater drainage systems, stormwater quickly flows into streams. This often causes elevated concentrations of nutrients and other pollutants, reduced habitat stability, and reduction of the aquatic species capable of inhabiting streams. As Danville has these attributes, the rapid fluctuation in water levels may be contributing to stream impacts.

2. Hanging Fork

As a result of the monitoring study, additional streams in the Hanging Fork watershed have been identified as impaired and listed on the 2008 303(d) list of impaired surface waters. Risk of disease due to human sewage and animal wastes is the most serious impairment to the watershed. Poor aquatic habitat is common throughout the watershed due to sparse vegetation surrounding streams. Each of the impacts identified in the monitoring are explained in more detail below.

Characterizing Physical Stream Impairments

- Habitat Assessment

Habitat assessment evaluates the physical structure of streams to determine how these factors influence water quality. Healthy streams provide diverse habitat for numerous species, and the assessment also determines the potential for the stream to provide habitat. The Kentucky Division of Water has established three categories to rank the quality of the habitat a stream provides, listed in the order of best to worst: "fully supporting," "partially supporting," and "not supporting."

Of the sixty-one sites surveyed in Hanging Fork, the majority of the sites were determined to have poor habitat. Although the poor habitat designations were due to different factors at various sites, common trends were observed in the watershed.

In the agricultural areas of the watershed, the poorest habitats frequently occurred in streams that pass through grazing areas. Cattle allowed to graze along the creek trample the banks and impact habitat by creating erosion that impacts aquatic habitat with sediment. Grazing also reduces habitat as cattle consume much of the streamside vegetation. Forested streams were generally in better condition than streams in agricultural areas.

In general, habitat of smaller streams and tributaries near the outer boundaries of the watershed, particularly in the southern portion, was much more impacted than on the larger Hanging Fork. Although the habitat of all streams in the watershed was not assessed, these trends appear representative of streams throughout the watershed.

The most negative impact to a stream's ability to provide habitat is due to the vegetated area adjacent to the stream, called the riparian zone, being either absent or underdeveloped. The riparian zone is important because it provides wildlife habitat, reduces stream erosion, filters nutrients, traps sediment, and provides canopy cover to the stream. Improving the riparian zone by vegetating the area within sixty feet of each stream bank with native plant species and reducing disturbance (human activity, livestock

Impact of Cattle Grazing in Hanging Fork

damage, etc.) will provide the greatest improvement to stream habitat.

- Pathogens

Results indicated that concentrations of *E. coli* often ranged from ten to one thousand times greater than the statewide limit. At their highest levels, some locations in the Hanging Fork watershed had *E. coli* levels similar to those found in the input to a wastewater treatment plant. Thus, the most significant impact in Hanging Fork is the fecal pollution of the watershed.

Overall, concentrations of *E. coli* were much higher in the southern portion of the watershed, averaging nearly double those found in the northern portion. Therefore, additional testing to identify the source of the fecal inputs and the relative concentrations of fecal bacteria in individual tributaries was focused in this area.

Despite the dominant agricultural land use of the watershed, human inputs were overwhelmingly shown to be the source of fecal inputs at the ten sites in which DNA testing was conducted. Generally, human inputs were found to contribute 75 percent of the fecal bacteria in the watershed. Cattle were identified as the second most abundant source, contributing 50 percent of fecal matter in some places, but averaging 25 percent or less. The source components in different geographical areas are shown in Exhibit 1.

DNA markers indicated that multiple residences throughout each watershed division are contributing to the high fecal levels. Testing to indicate the freshness of the fecal sources supports this conclusion. Since no residences outside of Junction City are serviced by sewer systems, failing septic systems and straight pipes are the dominant source of these high fecal levels. To a lesser degree, cattle contribute to the fecal impairment of the Hanging Fork watershed.

- Algal Blooms

Algal blooms are rapid increases in the stream's algal population that are caused by an abundance of nitrogen, phosphorus, and sunlight. Algal blooms were observed throughout the watershed but were especially abundant at Moores Lane. Concentrations of chlorophyll *a,* an indicator of algal blooms, were above the ecoregion average at all sites in which it was measured.

Algal blooms impact streams in a number of ways. The unattractive appearance can detract from the recreational value of the stream, causing property values to decline. Because of their volume, they also reduce habitat for some aquatic species. Algal blooms can also reduce nighttime concentrations of dissolved oxygen, which can be deadly to fish. Because dissolved oxygen was not measured at night, it is unknown whether the algal blooms are producing toxic conditions. However, no fish kills were observed in the watershed.

To reduce the occurrence of algal blooms, remediation should focus on reducing the input of nutrients (such as nitrogen and phosphorus) into the stream and increasing the riparian shading of the stream. By decreasing nutrient levels, less food is available to fuel abundant

algal growth. A vegetated riparian corridor with canopy cover will decrease the amount of sunlight reaching the stream and reduce the presence of algae.

3. Upper Dix

The upper Dix River watershed is primarily characterized by pasture agriculture combined with rural housing. As with the other subwatersheds, the primary impairments found were related to habitat degredations and elevated pathogens.

- Habitat

Overall, habitat scores in the Upper Dix River area were relatively good. Very little recent human channel alteration was observed at the sites. Streambanks appeared stable and well vegetated at most sites. As in the Hanging Fork and Clarks Run watersheds, the riparian zone widths are much smaller than desirable for optimal habitat. Because many of the sites have bedrock substrate, there is also less aquatic habitat available for macroinvertebrate colonization.

Of the nine sites in the Upper Dix River subwatershed, five sites were "fully supporting," two were "partially supporting," and two were "not supporting" their habitat use. Copper Creek, the lowest scoring site, was accumulating deposited sediment and becoming embedded. Habitat cover was reduced at Copper Creek, and the banks showed marginal stability and vegetative protection. The site with the best habitat rating in the entire watershed, Dix River at KY 52, contained a wide riparian width and optimal habitat scores for most categories.

- Pathogens

E. coli concentrations were lower overall in this watershed than in the Clarks Run or Hanging Fork watersheds, but exceedances of the acute water quality standard occurred regularly at all sites in the area. Drakes Creek had the highest concentrations and Gilberts Creek the lowest overall.

DNA analysis conducted at Drakes Creek, Logan Creek, and White Oak Creek identified the primary source contribution as human in each site, with estimations of 70 percent, greater than 70 percent, and 100 percent, respectively. This indicates failure in the area's sewage treatment facilities, both the municipal sewage treatment system and private septic systems, as the main source of these impacts.

- Chemical and Physiochemical

Nutrient levels at White Oak Creek, and to a lesser degree at Logan Creek, indicated excessive loading. White Oak Creek un-ionized ammonia results exceeded the regulated acute toxicity limit during only one month despite extremely high averages of nitrate and TKN levels at 5.5 mg/L and 2.0 mg/L respectively. Similarly, phosphorus levels at White Oak Creek were by far the highest in the project, averaging 1.25 mg/L for orthophosphorus and 1.48 mg/L for total phosphorus. Lancaster's wastewater treatment plant, located upstream of the site, is suspected as the primarily source of the nutrient loading.

Logan Creek, draining the city of Stanford, also had high nitrate and TKN levels but without un-ionized ammonia exceedances. Orthophosphorus was also high at 0.18 mg/L. Nitrogen and phosphorus results at other locations in the watershed showed more healthy levels for these compounds.

Suspended solids and turbidity readings were elevated at the larger sites on the Dix River at KY 52 and above the confluence of Hanging Fork, but also at smaller stream sites at Crab Orchard and Copper Creek. Siltation appeared to be impacting habitat, especially at Crab Orchard and Copper Creek, where slow flow rates allow sediment deposition.

Impacts to sensitive aquatic species may occur at White Oak Creek, Gilberts Creek, Logan Creek, and Drakes Creek due to elevated conductivity levels (Figure 24). White Oak Creek in particular had very high conductivity measurements, averaging 629 μS consistently. In the case of White Oak Creek, the abundance of nitrogen and phosphorus ions are heavily contributing to the conductivity of the stream.

B. Implementation

Implementation will be focused on the Clarks Run and Hanging Fork watersheds.

Recommendations for improving the aquatic impairments identified are multi-faceted. Best Management Plan (BMP) recommendations focus on the bacteria as well as the physical and chemical impairments in the watershed. The watershed plan indicates action items, organizations, funding, and indicators of success that may be used to ensure progress on these plans. The specific BMPs are listed below by impairment.

For *bacteria*, BMPs are recommended to reduce both human and livestock inputs.

For human inputs, specific BMPs include:

- Identifying and replacing failing and improperly maintained septic systems or straight pipes
- Identifying and repairing sewer collection system failures

For livestock:

- Restricting agricultural grazing from the riparian zone and installing filter strips to reduce fecal input from runoff

For elevated *nutrients*, the actions taken to address the bacterial sources should also reduce the nutrient sources. However, additional BMPs are necessary to reach the watershed goals including:

- Reducing WWTP limits on nitrogen and phosphorus
- Constructing headwater and streamside urban nutrient reduction features
- Constructing agricultural nutrient reduction BMPs

For *physical stream impairments*, efforts should focus on stream stabilization and riparian habitat expansion and establishment. In areas in which cattle are contributing to bacterial inputs, fencing cattle from the stream and allowing the riparian vegetation to establish may accomplish two goals.

Other specific tasks include:

- Planting riparian trees to increase riparian vegetated width
- Reduce stream flashiness by reducing or slowing stormwater runoff
- Stream restoration
- Rain barrel installations
- Increase enforcement of existing ordinances and regulations

Other BMPs should focus on *litter* and *public education*. Specific efforts should include:

- Enforcement of litter and dumping ordinances
- Conducting community trash pickup days
- Increasing public education by increasing accessibility to water quality related information
- Encouraging community interest in stream improvement
- Examining and recommending updates to local codes and ordinances

The following Tables 8 and 9 give specific information regarding the specific objectives, BMPs, and the Action Items associated with each.

TABLE 8 – BEST MANAGEMENT PRACTICES AND ACTION ITEMS, CLARKS RUN WATERSHED

OBJECTIVE	BMP	ACTION ITEMS
#1: Reduce human fecal inputs from septic tanks and sewer exfiltration	1) Identify and replace failing and improperly maintained septic systems or straight pipes	1) Field scouting to identify illicit discharges from straight pipes and to identify and confirm the numbers and locations of failing septic systems
		2) Notify approximately 15 landowners and health department of field confirmed failing septic systems to allow for correction or enforcement
		3) Educate community on septic tank maintenance and indicators of poor performance through distribution of the "Homeowner's Guide to Septic Systems" and household mailer
		4) At least 15 septic systems will be rehabilitated in Balls Branch West, Balls Branch Mouth, US 127 Bypass, and Corporate Drive watershed areas. Others identified by field surveys addressed based on availability of funding
	2) Identify and repair failures in the sewer collection system	1) City of Danville to work in conjunction with citizen volunteers and monitoring groups to increase watershed scouting for sanitary sewer exfiltration and illicit storm sewer connections using *E. coli* and conductivity monitoring in the Stanford Road and South Second Street watershed reaches
		2) Continue in-line video inspections of sanitary sewer systems and pressure checks to target maintenance
		3) Hotline for pollution prevention and notification with a link on the website to allow homeowners to report illicit discharges in the area
		4) Identify funding for sewer system repairs
#2: Reduce fecal inputs from livestock	3) Restrict agricultural grazing from the riparian zone and install filter strips to reduce fecal input from runoff	1) Host a workshop or presentation on water quality issues and cost share programs at the Cattleman's Association and other agricultural organizations 2) Develop a list of landowners with the largest portions of stream for targeted encouragement to improve riparian shading, vegetation, or fencing. 3) Utilize NRCS Cost Share practices for fencing (Practice #382), livestock exclusion (#472), and filter strip (#393) as well as other reduction alternatives
#3: Reduce algal blooms and eutrophication by decreasing nitrogen and phosphorus loading	4) Reduce WWTP limits on nitrogen and phosphorus	Establish discharge limits on Danville's WWTP such that the TMDL targets for phosphorus (0.3 mg/L) and nitrogen (2.0 mg/L) are met
	5) Construction of headwater and streamside urban nutrient reduction features	Utilize Stormwater fund to direct the construction of urban nutrient reducing BMPs such as grassy swales, rain gardens, streamside wetlands, and other applicable infrastructure to the watershed between Stanford Rd to Corporate Dr.

	6) Construction of agricultural nutrient reduction BMPs	Target landowners in Balls Branch and Corporate Drive/ US127 for the use of NRCS practices such as fencing, filter strips, animal waste control, riparian buffers and other nitrogen reduction techniques
#4: Increase riparian vegetated width	7) Conduct riparian tree planting in rural areas	Utilize NRCS Cost share practices for riparian forested buffer (#391) and tree planting (#612)
#5: Reduce the stream flashiness by reducing or slowing stormwater runoff	8) Stream restoration on some particularly eroded or impaired locations	Identify, design, and implement stream restoration on impaired reaches
	9) Encourage the use of rain barrels to reduce runoff volume	Establishment of a rain barrel distribution program similar to Lexington's "Lily Program" to reduce stormwater runoff
	10) Increase enforcement of ordinances and regulations	Enforce erosion control Ordinances and stormwater permit post construction program
#6 Reduce litter in streams	11) Enforce litter and dumping ordinances	Post signs at Goggin Lane Overpass and along the pull off at Mansfield Road indicating the penalty for littering
	12) Conduct community trash pickup days	Organize community pickups along known areas of littered streams
#7: Increase knowledge of water quality issues such that citizens and local officials can address impairments with appropriate codes, ordinances, and other practices	13) Increase public education by increasing accessibility to water quality related information	1) Develop an environmental resources display for the Boyle County Public Library and host an education event
		2) Incorporate Bluegrass PRIDE's water quality education curriculum at local elementary and middle Schools
		3) City Council has appointed a commission to incorporate trail systems into the City of Danville's Master Plan. Utilize the Trail System integration into the riparian zone to increase public awareness of Clarks Run
		4) Link the Danville's Stormwater website to the Dix River Watershed webpage as well as CREEC and other watershed organizations to increase access to the watershed based plan and citizen action opportunities
		5) Utilize the GreenTips section of the local paper to publish results of watershed plan and how homeowners can improve water quality in their area
	14) Encourage community interest in stream improvement	1) Post signage throughout the watershed at trail systems and overpasses identifying the streams, watershed boundaries, and water quality information
		2) Organize a World Water Monitoring Day to gain interest of community children in the water quality of the Clarks Run watershed
		3) Provide a workshop to familiarize developers with improved techniques for low impact development
	15) Examine and recommend updates to local codes and ordinances	1) Revision of the Stormwater Manual to include more effective water quality ordinances with new MS4 permit 2) Recommendations from Bluegrass PRIDE's ordinance manual to be incorporated where relevant

TABLE 9 – BEST MANAGEMENT PRACTICES AND ACTION ITEMS, HANGING FORK WATERSHED

OBJECTIVE	BMP	ACTION ITEMS
#1: Reduce human fecal inputs from septic tanks	1) Address failing and improperly maintained septic systems	1) Field identification of approximately 307 failing systems outside of the proposed sewer corridor in Blue Lick (38), McCormick Church and Chicken Bristle (93), Peyton Creek (49), McKinney Branch (91), and West Hustonville (36) watershed areas
		2) Notify approximately 307 landowners and health department of field confirmed failing septic systems to allow for correction or enforcement
		3) Educate community on septic tank maintenance and indicators of poor performance through distribution of the "Homeowner's Guide to Septic Systems" and household mailer
		4) Rehabilitate 307 failing systems identified by field surveys
	2) Replace septic systems with a sanitary sewer collection system	1) Remove over 1,250 septic systems through an extension of Danville's sanitary sewer collection system to the Hustonville/Moreland area
		2) Write letters to local officials and newspaper articles encouraging the construction of a package plant in the McKinney area to address high density of failing septic systems
#2: Reduce fecal inputs from livestock	3) Restrict agricultural grazing from the riparian zone	7) Host a workshop or presentation on water quality issues at the Cattleman's Association and other agricultural organizations 8) Develop a list of landowners with the largest portions of stream for targeted encouragement to improve riparian shading, vegetation, or fencing 9)Utilize NRCS Cost Share practices for fencing (Practice #382), livestock exclusion (#472), filter strip (#393), riparian forested buffer (#391) and tree planting (#612)
	4) Install filter strips along waterways to reduce fecal input from runoff	
#3: Increase the stream shading	5) Conduct riparian tree and shrub planting	
#4: Increase riparian vegetated width	6) Conduct re-vegetation of riparian width through mowing restrictions and plantings	
#5: Increase knowledge of water quality issues such that citizens and local officials can address impairments with appropriate codes, ordinances, and other practices	7) Hire a local water quality advocate for planning decisions	10) Utilize the Office of Surface Mining VISTA program to acquire a watershed coordinator
	8) Increase public education by increasing accessibility to water quality related information	11) Develop an environmental resources display for the Lincoln County Public Library and host an education event
		12) Organize a minimum of 2 annual radio announcements, 3 newspaper editorials, and personal communication with 100 landowner interactions about watershed impairments and BMPs
	9) Encourage community interest in stream improvement	13) Encourage Hustonville Elementary, McKinney Elementary, and Lincoln County Middle and High Schools to utilize Bluegrass PRIDE K-12 water quality curriculum
		14) Install signage along roadways and parks identifying streams and water quality issues
		15) Sponsor KRWW volunteer monitoring of subwatershed areas
		16) Identify greenspace areas for public parks along creek and outdoor classroom areas
	10) Examine and recommend updates to local codes and ordinances	17) Develop local codes and ordinances to reduce the impact on riparian areas
		18) Encourage the county and cities to use water quality modeling in making planning decisions

C. Discussion

Significant amounts of water quality data collected in the Dix River watershed have highlighted significant amounts of pollutant sources. At present, the greatest stressors in the watershed are human fecal contributions, high nutrient outputs from the WWTP, cattle access to stream riparian areas, and high velocities of water from urban impervious surface. The current establishment of the Stormwater Management Fund shows promise towards reducing nitrogen inputs as well as decreasing the velocity of stormwater entering Clarks Run. The strong involvement of local watershed and environmental groups such as CREEC, Healthy Planet Initiatives, Herrington Lake Conservation League, and KRWW show broad-based community interest and support of water quality improvements. In addition, the relationship between Centre College and these groups provides a large volunteer base for watershed projects.

The elimination of problems in the sewer collection system will remain a challenge for future watershed work. Obvious overflows and leaks have been detected through in-line video and field scouting; however, exfiltration sources are more difficult to detect.

Cattle production will continue to be a dominant land use in the rural portions of the watershed. Decreasing the detrimental influence of cattle grazing on stream habitat and water quality is currently a challenge and will continue to be one in the future. Encouraging participation of local farmers in cost share programs is often difficult without increased incentives.

Educational activities should increase the knowledge of water quality issues such that citizens and local officials can address impairments with appropriate codes, ordinances, and other practices. Specific actions should include:

- Hire local water quality advocate for planning decisions
- Increase public education by increasing accessibility to water quality related information
- Encourage community interest in stream improvement
- Examine and recommend updates to local codes and ordinances

IV. CONCLUSIONS

The information presented in this document and those that preceded it document the impairments in the Dix River watershed and also give a clear recommendation for improvement. In general, high *E. coli* concentrations and narrow riparian vegetation width are the main impairments in the Dix River watershed. Because testing indicates that the high *E. coli* concentrations are due completely to human inputs in some areas, these high concentrations represent not only stream impairment but also a health threat. Remediation will be a considerable challenge, but the information provided from this study will provide leverage for identifying support and funding for the necessary changes.

The overall goal of the Dix River/Herrington Reservoir Clean Water Action Plan is to reduce nonpoint source pollution and improve the bacterial and biological integrity of Herrington Reservoir and the streams within the Dix River watershed. The studies presented here did not accomplish this task specifically but did provide base information that will be used to

determine measure improvements in the watershed. These studies also presented clear action items to facilitate the improvements.

Specific objectives of the project were:

1. Encourage project participation and a holistic approach by involving a variety of agencies, organizations and citizens groups in the implementation of the project
2. Develop a holistic and realistic plan for the restoration of the Dix River/Herrington Reservoir watershed
3. Encourage implementation of the watershed-based plan

Objective 1 was accomplished through the involvement of multiple watershed group meetings. Objective 2 was accomplished through the completion of watershed-based plans for Clarks Run and Hanging Fork watersheds. Objective 3 is currently being accomplished through additional implementation efforts.

This project also provided many lessons which could be used to improve future water quality analyses. Some of these lessons include the following:

- **Importance of flow measurements**

The importance of consistency in measuring flow was underscored throughout the project. Loading calculations are dependent on two variables, flow and the concentration of the pollutant of concern. Although the quality of laboratory data is typically well documented in the project QAPP, the quality objectives for flow measurements are more difficult to establish and are often more nebulous. Different methods of measuring flow including the General oceanic current meter, the Marsh-McBirney Doppler velocity meter, and the "floating object method" each yielded differing results when tested under the same conditions. Currently no published data was identified indicating the variability associated with each method and the comparability of each method with other methods. Such research would greatly aid in improving consistency in these measurements.

- **Laboratory detection limits**

Although the laboratories met the project specified detection limits for nutrient data, the calibration curve caused results to be biased low as levels approached the reporting limit. For methods in which the accuracy of data near the detection limit is critical, the QAPP should require laboratory analysis of a laboratory control sample near the detection limit in order to evaluate the accuracy at these low concentrations.

- **Loading calculations**

Through the analysis of the data in preparation of watershed plans, the need for a standardized method of calculating pollutant loadings was highlighted during this study. Loading calculations differed depending on the type of averaging utilized (i.e. arithmetic average versus geometric average) and whether the averaging was conducted before or after the flow and pollutant concentrations were multiplied for each sampling visit. Because future monitoring data will be used to evaluate progress in achieving water quality goals, methods for calculating loading should be standardized to maximize the comparability of measurements.

- **Microbial Source Tracking Limitations**

The microbial source tracking methods utilized in this study provided crucial information about the source of the fecal pollution. The relative contributions of human and cattle sources were successfully characterized in sufficient detail to focus remediation efforts towards the most significant sources. However, the number of samples collected and the accuracy of the qualitative analyses were not sufficient to indicate the contribution of wildlife sources to the fecal loading. In future studies were such information is more crucial, microbial source tracking methods specific to wildlife fecal sources should be utilized.

REFERENCES

Carey, D.I. *et al.* 1993. Quality of Private Ground-Water Supplies in Kentucky. Kentucky Geological Survey. Information Circular 44 Series XI. ISSN 0075-5583.

Carey, D.I and Stickney, J.F. 2004. Groundwater Resources of Boyle County, Kentucky. County Report 11, Series XII. Kentucky Geological Survey. ISSN 0075-5567.

Carrier, Randy and Metcalfe, Jack. Personal communication on July 12, 2007. During meeting project area maps were reviewed by Lincoln County Health Department staff and data was later converted to GIS files.

City of Danville. 2006. Stormwater Manual.

Halcomb, Larry and Stevens, Jason. Personal communication on July 18, 2007. During meeting project area maps were reviewed by Boyle County Health Department staff and data was later converted to GIS files.

Horsley & Whitten. 1996. Identification and Evaluation of Nutrient and Bacteriological Loadings to Maquoit Bay, Brunswick, and Freeport, Maine. Final Report. Casco Bay Estuary Project, Portland, ME.

Howard K Bell Consulting Engineers. 2006. 201 Facilities Plan Update for the City of Danville, Boyle County, Kentucky.

Kentucky Division of Water (KDOW). 1990. Section 303(d) List of Waterbodies for Kentucky. Robert W. Ware. Water Quality Branch. Division of Water. Natural Resources and Environmental Protection Cabinet.

KDOW. 2007. Kentucky Consolidated Groundwater Database. Accessed 2009.

KDOW. 2008a. Final 2008 Integrated Report to Congress on the Condition of Water Resources in Kentucky: Volume II 303(d) List of Surface Waters. Kentucky Environmental and Public Protection Cabinet Division of Water.

KDOW. 2008b. *Methods for Assessing Biological Integrity of Surface Waters in Kentucky.* Kentucky Department of Environmental Protection.

Kentucky State Nature Preserve Commission (KSNPC). 2009. Report of Endangered, Threatened, and Special Concern Plants, Animals, and Natural Communities for Boyle County, Kentucky. Current as of February 2009. http://www.naturepreserves.ky.gov/

Metcalf and Eddy. 1991. *Wastewater Engineering: Treatment, Disposal, Reuse*. 3rd Edition. McGraw-Hill, Inc., New York.

Midwestern Regional Climate Center (MRCC). 2009. Historical Climate Data. Station: 152040 Danville, KY. Accessed online at http://mcc.sws.uiuc.edu/climate_midwest/mwclimate_data_summaries.htm#

Morgan, Josh. Personal communication on July 14, 2009.

NRCS. 2003. National Resources Conservation Service Conservation Practice Standard CODE 612: Tree/Shrub Establishment. NRCS Kentucky.

NRCS. 2006. Garrard Lincoln Counties Soil Survey, Kentucky

Ray, J.A. J.S. Webb, P.W. O'dell. 1994. Groundwater Sensitivity Regions of Kentucky. Kentucky Department of Environmental Protection Division of Water Groundwater Branch. http://kgsweb.uky.edu/download/wrs/sensitivity.pdf

Ray, J.A. 2002. Cryptic Artesian Flow of Bull Spring: A Karst Rarity. Oral Presentation: Geological Society of America Southern Section Meeting. Lexington, KY.

Renfro, Bo. Lincoln and Garrard County NRCS Agent. Personal communication concerning BMP costs. August 2009.

Robson, M. B. 2006. Memorandum concerning "Division of Water File Review" submitted to Gerry Fister, Third Rock Consultants.

Roessler, Rose-Marie, 2006. Bioassessment of the Water Quality of Clarks Run, Boyle County, Kentucky Using Chemical Parameters and Macroinvertebrates as Biological Indicators. Masters Thesis. Eastern Kentucky University.

US Department of Agriculture/NRCS, 2007a. Ky SSURGO Soils Status Map 01-2007. Updated January 27, 2007. Accessed April 23, 2007.

US Department of Agriculture/NRCS, 2007b. Excerpts from Soil Properties Report Descriptions at http://soildatamart.nrcs.usda.gov/Report.aspx. Accessed May 5, 2007.

United States Environmental Protection Agency (USEPA). 2006. STORET database. Accessed on August 2006, for all surface water quality data collected by Kentucky Division of Water through August 2006.

USEPA. 2009a. Website: "Water Quality Criteria for Nitrogen and Phosphorus Pollution." Download by State of Kentucky. http://oaspub.epa.gov/nutrient/download.download_state?state_abbr=KY Accessed September 1, 2009.

USEPA. 2009b. Website: "EPA> OWOW> Monitoring and Assessing Water Quality> Volunteer Stream Monitoring: A Methods Manual>Chapter 5> 5.9 Conductivity." http://www.epa.gov/volunteer/stream/vms59.html Accessed September 1, 2009.

United States Geological Survey (USGS). 1999. Modeling Hydrodynamics and Water Quality in Herrington Lake, Kentucky. Water-Resources Investigations Report 99-4281. In cooperation with the Kentucky Natural Resources and Environmental Protection Cabinet

Woods, A.J., Omernik, J.M., Martin, W.H., Pond, G.J., Andrews, W.M., Call, S.M, Comstock, J.A., and Taylor, D.D., 2002, Ecoregions of Kentucky (color poster with map, descriptive text, summary tables, and photographs): Reston, VA., U.S. Geological Survey (map scale 1:1,000,000).

APPENDICES

Appendix A – Financial and Administrative Closeout

The budget summary for this project is shown below.

BUDGET SUMMARY FOR DIX RIVER
DETAILED BUDGET INFORMATION

Budget Category	319(h) Dollars	Match	Total	Final Expenditures
Personnel				
Supplies				
Equipment				
Travel				
Contractual-RFP	$234,959	$156,639	$391,598	$391,598
MST contract	$37,169	$24,780	$61,949	$61,949
Change Order	$153,744	$102,496	$256,240	$256,240
Operating Costs				
Other				
Total	$425,872	$283,915	$709,787	$709,787

Third Rock Consultants, LLC was competitively hired following the Commonwealth of Kentucky's procurement process for all 3 projects. Third Rock Consultants, LLC has been paid a total of $450,652 as follows: $425,872 in federal funds and $24,780 in state funds.

Application Outputs

Microbial Source Tracking Report
July 18, 2008

Dix River Monitoring Report
March 1, 2009

Clarks Run Watershed Brief
July 15, 2009

Hanging Fork Watershed Brief
July 15, 2009

Clarks Run Draft TMDL
September 14, 2009

Clarks Run Watershed Plan
November 2, 2009

Hanging Fork Watershed Plan
November 2, 2009

APPENDIX B – Quality Assurance Project Plan: Monitoring, Assessment, and TMDL Development for the Dix River Watershed (August 2006)

Quality Assurance Project Plan

Monitoring, Assessment, and TMDL Development for the Dix River Watershed

Prepared for
Kentucky Environmental and Public Protection Cabinet
May 17, 2006
Revised August 30, 2006

Prepared by
Third Rock Consultants, LLC
2514 Regency Road
Lexington, KY 40503
859.977.2000
www.thirdrockconsultants.com

Quality Assurance Project Plan

Monitoring, Assessment, and TMDL Development for the
Dix River Watershed

for

Kentucky Environmental and Public Protection Cabinet
Department for Environmental Protection
Division of Water
14 Reilly Road
Frankfort, KY 40601

May 18, 2006
Revised August 30, 2006

THIRD ROCK CONSULTANTS

www.thirdrockconsultants.com

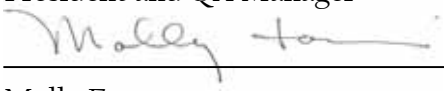
Environmental Analysis & Restoration

Distribution and Review List

Quality Assurance Program Plan for Dix River Watershed
Revision: 1, Dated: August 30, 2006

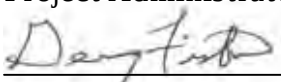
1) Third Rock Consultants, LLC

President and QA Manager



Molly Foree — August 30, 2006 (Date)

Project Administrator

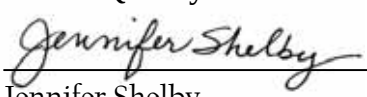


Gerry Fister — August 30, 2006 (Date)

Data Manager

Marcia Wooton — August 30, 2006 (Date)

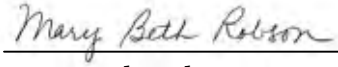
Water Quality Modeler



Jennifer Shelby — August 30, 2006 (Date)

2) GRW Engineers, Inc.

Water Quality Modeler



Mary Beth Robson — August 30, 2006 (Date)

3) CT Laboratories

Laboratory Director

David Berwanger — August 30, 2006 (Date)

TABLE OF CONTENTS

APPENDICES – FIGURES AND TABLES

1. Project Management

1.1 Introduction

This Quality Assurance Project Plan (QAPP), prepared by Third Rock Consultants, LLC (Third Rock), was approved by the Kentucky Division of Water (KDOW). This QAPP covers the planning, implementation, and assessment procedures necessary to meet the minimum data quality objectives (DQOs) for the monitoring, assessment, and TMDL development for the Dix River Watershed, Kentucky.

Third Rock is committed to producing quality data that will assist the Division of Water in the development of their watershed plan. This QAPP is designed to provide a complete plan for achieving all project data quality objectives. However, effective communication is required to ensure all parties properly implement the plan. Any quality feedback, questions, or concerns related to the project should be communicated to the project administrator or quality manager to facilitate appropriate analysis and resolution.

1.2 Project Organization

1.2.1 Kentucky Division of Water, Primary Data User

The monitoring, assessment, and TMDL development activities conducted by Third Rock Consultants, LLC for the Dix River Watershed will be under the jurisdiction and oversight of the Kentucky Division of Water (KDOW) Watershed Management Branch. Lee Colten serves as the KDOW Project Manager, providing overall direction and guidance to the project. Third Rock's project administrator will communicate directly with Mr. Colten to ensure that all project objectives are satisfied.

Eric Liebenauer serves as the KDOW Water Quality Modeler. In this capacity, he provides guidance for Third Rock's Water Quality Modeling for Clark's Run and will perform the modeling for the Hanging Fork based on the data provided by Third Rock.

1.2.2 Third Rock Personnel and QA Responsibilities

The implementation of the project plan requires effective operation of the project team. Figure 1, Dix River Organizational Chart, identifies the parties that comprise the Dix River Project Team and the lines of authority and communication under which this team operates. The specific roles and responsibilities of each key party are documented below.

- ***Project Administrator***

Gerry Fister will serve as the Project Administrator. Mr. Fister is responsible for the overall completion of the project to the requirements of the KDOW. In this capacity, he is responsible for overall project administration, personnel, scheduling, and completion of all data quality objectives. Additionally, he maintains project financials and contracts and submits reports to the KDOW. Mr. Fister serves as the primary contact with the Kentucky Division of Water.

- ***Field Logistics Coordinator***

Tony Miller will serve as the field logistics coordinator. Mr. Miller visually assessed the watershed for nonpoint source pollutants and determined site selection per the TMDL modeling requirements. He additionally researched and built the equipment associated with the Periphyton sampling. Mr. Miller is responsible for report generation, internal technical assistance, and public communications.

- ***Water Quality Modelers***

Jennifer Shelby in conjunction with Mary Beth Robson of GRW Engineers will serve as the Water Quality Modelers. Together they are responsible for the TMDL modeling of the Clark's Run load allocation and training of the KDOW on modeling calibration, application, and manipulation. In the modeling capacity, they are responsible for selection and setup of the modeling reaches, setup of modeling climate, calibration of the model for all parameters, preparation of the modeling summary, and

selection of sensitivity scenarios. As trainers, they are responsible to enable the Division of Water staff to evaluate the effects of the new nutrient criteria on the load allocations.

- ***Quality Assurance Manager***

Molly Foree will serve as the Quality Assurance Manager. Ms. Foree is responsible for review of the QAPP, field operations procedures, and data documentation procedures that will help ensure field and laboratory data generated meet data quality objectives. Ms. Foree will remain independent of the data collection. She is responsible for the maintenance and distribution of the approved QAPP.

- ***Data Manager and Sampling Coordinator***

Marcia Wooton will serve as the Data Manager and Sampling Coordinator. Ms. Wooton is responsible for the review of laboratory analytical results and coordination of sampling events. As sampling coordinator, she is responsible to ensure that the sampling procedures and schedule is implemented by the sampling technicians. Ms. Wooton communicates with the laboratories to ensure holding requirements and other data quality objectives are met. Additionally, she notifies the laboratory of sampling bottle preparation needs. As Data Manager, Ms. Wooton reviews analytical data generated by the laboratory and the field, including the COMPASS tables, and ensures that it conforms to the requirements of this QAPP.

- ***Sampling Technicians***

Cory Bloyd will serve as the Primary Sampling Technician with the support of John Davis, Dan Miller, Tony Miller, Johnny Varner, and Steve Evans. Sampling Technicians are responsible for implementing the sampling procedures and schedule as coordinated by the Data Manager and Sampling Coordinator.

1.2.3 Subcontractor Responsibilities

1.2.3.1 CT Laboratories of Baraboo, Wisconsin

The analytical subcontractors for the laboratory portion of this project will be CT Laboratories of Baraboo, Wisconsin for all laboratory parameters except Total Coliform / *E. coli* which will be provided by Microbac Laboratories of Lexington, Kentucky. The laboratory will be responsible for analysis of samples delivered such that data quality objectives are met. The laboratory will implement and document QA/QC activities to support the results of the analyses performed on the samples. All analyses are expected to be conducted in accordance with the specified analytical methods, the laboratories QA manual, and this QAPP. Eric Korthals, laboratory project manager, is responsible for ensuring conformance of the laboratory.

The following provides a general summary of the QA responsibilities of key laboratory personnel:

- ***Laboratory Director***

David Berwanger will serve as the Laboratory Director for CT Laboratories. The Laboratory Director is responsible for the supervision of all functional aspects of the laboratory and has authority in a legally binding capacity for all laboratory decisions and operational issues. Responsibilities may include, but

are not limited to, overseeing personnel training, equipment and systems maintenance, laboratory safety, monitoring scheduling and status of work, approval of Standard Operating Procedures, implementing preventive and corrective actions, and cost control. The Laboratory Director is responsible for ensuring laboratory personnel implement internal lab QA/QC procedures and comply with applicable regulations.

- *Laboratory Quality Assurance Director*

Dan Elwood will serve as the Laboratory Quality Assurance Director for CT Laboratories. The Laboratory Quality Assurance Director has authority over and is responsible for the direction of all laboratory QA activities, and is independent of laboratory production functions. The Laboratory Quality Assurance Director's responsibilities include development, documentation, and evaluation of quality assurance/quality control (QA/QC) procedures and policy. He/she conducts internal audits, reviews data reports, compiles and evaluates method performance, trains staff in QA/QC requirements, tracks non-conformances and corrective actions, prepares quality documents and reports, reviews standard operating procedures, and reports findings and quality issues to the Laboratory Director. A primary responsibility of the Quality Assurance Director is to verify that all personnel have a clear understanding of the QA program, know their roles relative to one another, and appreciate the importance of their roles to the overall success of the program.

- *Laboratory Information System Managers*

David Berwanger and Jason Remley will serve as the Information Systems (IS) Managers for CT Laboratories. The IS Manager's responsibility includes development and maintenance of the software and hardware components of laboratory operations. He/she ensures all systems are operating and validates any computer programs involved in the data reduction, generation and reporting process. The IS Manager serves as the database administrator for the Laboratory Information Management System(LIMS). The IS Manager is responsible for producing data in COMPASS format for this project.

- *Laboratory Project Manager*

Eric Korthals will serve as the Laboratory Project Manager for CT Laboratories. Project Managers are the Third Rock's primary point of contact for laboratory analytical services. The Laboratory Project Manager's duties involve performing as a client-laboratory liaison for project work, working with customers to identify project-specific requirements, and aiding them, throughout the laboratory, to meet their data quality objectives. Project managers review analytical results to ensure project data and QC requirements have been satisfied, prepare narrative reports where applicable, and monitor project work so deadlines are met. They are responsible for seeing that clients are informed of any quality problems as soon as possible. Project Managers work directly with the laboratory managers and laboratory staff involved in their assigned projects to keep staff informed of QA/QC requirements and to monitor work progress. They also work closely with Third Rock and KDOW to develop work plans and DQOs for current and future work.

1.3 Problem Definition and Background

Herrington Lake, in the Kentucky River Basin, was formed by the impoundment of the Dix River. As is common with many reservoirs, Herrington Lake is subject to excessive nutrient loading resulting from point and nonpoint source contributions within the watershed. The Dix River watershed has 24 permitted wastewater-discharge sites and Herrington Lake directly receives wastewater from 6 of the 24 wastewater-discharge sites. In addition, the Dix River watershed contains failing septic systems, agricultural activities including numerous cattle with free access to streams, and development / construction activities. This abundant nutrient input has lead to the deterioration of water quality, problematic algal blooms, and subsequent fish kills.

Herrington Lake was listed in the 2004 303(d) report as 1st priority impaired waterbody for aquatic life (non-support) and fish consumption (partial-support). The major tributaries to the reservoir, Dix River, Clarks Run, and Hanging Fork, were also cited in the 2004 303(d) report as having segments listed as 1st priority impaired in regards to aquatic life support and primary contact (non-support and partial support). The cited reasons for impairment are primarily low levels of dissolved oxygen (DO) and high levels of bacteria. Sources of both impairments stem from agricultural runoff, septic-tank leakage, urban/suburban stormwater runoff, and wastewater treatment plant (WWTP) discharges (USGS 2000).

As part of KDOW's 1998 Clean Water Action Plan, the Natural Resources Conservation Service (NRCS) and KDOW jointly selected five priority watersheds in Kentucky for targeted water quality improvement. The Dix River was selected as one of these priority watersheds. KDOW has committed to form a watershed council to provide input on watershed analysis and plan development. Between 2006 and 2007, KDOW intends to:

- Develop TMDLs for subwatersheds of the Dix River including Clarks Run, Hanging Fork and Herrington Lake (a TMDL, or Total Maximum Daily Load, identifies pollutant sources and the amount of pollutants from each source, and makes recommendations for pollutant loads a stream can handle without violating water quality standards).
- Develop a watershed plan to reduce pollutants from point and non-point sources
- Identify funding sources to implement practices that can reduce pollutants
- Present a draft watershed plan to the watershed council and various stakeholders, and
- Begin implementing remediation actions identified in watershed plan

In order to assist the KDOW in meeting these goals, Third Rock Consultants, LLC has been contracted to identify nutrient and bacteria sources throughout the Dix River watershed and conduct a modeling study in support of a TMDL for nutrients and dissolve oxygen for Clarks Run. Additionally, KDOW will calculate a TMDL for bacteria for Hanging Fork from data provided by the Third Rock sampling effort.

1.4 Project Description

1.4.1 Summary

Third Rock Consultants' ultimate goal coincides with the Kentucky Division of Water: to remove the tributaries upstream of Herrington Lake (and ultimately Herrington Lake) from the 303(d) list of impaired streams by providing information that will focus water quality improvement actions.

In order to accomplish this goal, specific project tasks of Third Rock are as follows:
1. Identify sites for monitoring on the Dix River watershed that includes Clarks Run and Hanging Fork
2. Perform monitoring and laboratory analysis of the Dix River Watershed providing provide high quality water data for the purpose of determining the source and extent of impairment in the tributaries of Herrington Lake
3. Prioritize sources of impairments and develop a TMDL modeling study for nutrients and dissolved oxygen on Clarks Run.
4. Provide training to KDOW staff on TMDL model
5. Generate ideas for non-point source solutions

Figure 2, Dix River Project Schedule, in the appendix, provides the scheduled time period over which these objectives are expected to be achieved. In general, the sampling effort will last twelve calendar months followed by a 90-day modeling effort and modeling report composition. Additionally, Third Rock will provide continued support to the DOW after TMDL modeling with the further development of allocations, load reductions, and an implementation plan. For each of the goals specified above, a summary of the tasks associated with accomplishing each goal is presented in more detail in the following sections.

1.4.2 Site Identification and Preparation

Prior to the establishment of monitoring locations, all major reaches in Clarks Run and Hanging Fork (Hydrologic Unit Level 14 Code (HUC14) and smaller) were visually surveyed to optimally locate sampling stations relative to nonpoint and point source contribution. The sites were marked with GPS waypoints and photographed.

Site locations on the Dix River, Clarks Run, and Hanging Fork were chosen by Third Rock in conjunction with KDOW to characterize the dissolve oxygen, nutrients, sediment, and coliform loadings and to facilitate modeling of these parameters. Sites are located downstream of known problem areas to quantify potential pollutant contribution. Two types of sampling sites are located in the watershed, *select* and *non-select* stations.

Non-select stations

Non-select stations are sampled during low, normal, and high flows. Permanent monuments (survey pins) were established to standardize water collection, flow measurement, and photograph locations at

each station. Cross-section measurements were completed at each station to support discharge computation. For each cross-section, three reference points were established. Two of the points, located on opposite sides of the bank, were located for subsequent section measurements. The third point will be located for reference of stage readings. Stage reference points may be located on a bridge, established with pins (rebar), or a sturdy overhanging limb. Water samples will be collected from all identified stream stations throughout the entire watershed according to the monthly field schedule prepared by the Data Manager and Sampling Coordinator.

Select stations

All sampling and preparation that applies to non-select stations also applies to select stations with the addition of several parameters. Select stations additionally have a stormwater sampling component. Passive high flow samplers will be used to assess the peak nutrient and bacterial contribution during heavy rainfall events. Passive high flow sampling device locations will be determined and installed by October 2006. Select stations will also sampled for additional analytical parameters (see Table 1). Six select stations will additionally be mounted with continuous monitoring pressure transducer water level recorders; Drakes Creek, Dix Above, Knob Lick, Hanging Fork 150, Clarks Run Bypass, and Balls Branch Mouth.

The locations of all sampling stations are mapped on either Figure 3, Watershed Overview Map; Figure 4, Hanging Fork and Clarks Run Map; or Figure 5, Dix River Map found in the appendix. For each subwatershed, the following summarizes the station locations and considerations in their establishment.

Clarks Run

Eight sites (four select and four non-select) in the Clarks Run subwatershed were established.

Hanging Fork

In the Hanging Fork watershed, fourteen stations (six select and eight non-select) were established.

Dix River

Seven stations (one select and six non-select) in this section of the watershed were located upstream of the Hanging Fork convergence with the Dix River.

1.4.3 Monitoring

Monitoring, which includes, field observations and measurements, provide data valuable for water quality assessment and modeling. Field sample collection directly affects the analytical results generated by the laboratories. Effective monitoring is essential to determining the source and extent of the impairments in the tributaries of Herrington Lake and Dix River Watershed.

For twelve months, monthly g*rab samples* will be taken at *all sampling stations and analyzed* as listed in Table 1, Sample / Results Summary for Dix River Watershed. Grab samples from all sites are collected for laboratory analysis for total and ortho-phosphorus, nitrate and nitrite, total kjeldahl nitrogen, ammonia, total organic carbon (TOC), total suspended solids (TSS), total coliform and *E. coli*. Field measurements for dissolved oxygen, temperature, conductivity, flow, and pH will be made at all sites as well.

In addition to these parameters, some sites will have further analysis. The Hanging Fork select stations and all Clark Run stations will be analyzed for 5-day biochemical oxygen demand (BOD5) for the dissolved oxygen modeling. Also, grab samples from the Clarks Run select stations will be analyzed for 15-day BOD. Chlorophyll *a* and alkalinity will be collected monthly and chlorides quarterly for all select stations.

Sampling events for these collections shall coincide adequately with high, low, and medium flow events. The high-flow samples at the *select stations* will be collected using the *passive high flow sampling* for all of the above chemical parameters. Sampling periods will coincide with elevated flow from November to April with a goal of capturing one high flow event per month following a seven day dry period. The schedule will also be managed to ensure that low and medium flow events are captured. Methods for passive high flow sampling will consist of a low-tech sampler based on methods presented in Subcommittee on Sedimentation, 1961. Sample bottles are mounted on an in-stream frame and filled as the stream rises. Once the stream recedes samples will be collected for analysis.

During the recreational period (May – October), Third Rock will dispatch sampling technicians to collect samples from Hanging Fork during a high flow period. Because the passive high flow samplers would bias total coliform and *E. coli* results, technicians will be in the watershed as the storm event occurs to allow collection of these samples during the hydrographic rise of the stream. This storm event should occur after a relatively dry period.

Periphyton: Periphyton will be collected from natural substrate at the select stations and measured from chlorophyll *a* and multihabitat samples. Chlorophyll *a* will be collected by agitating 0.25m^2 of natural substrate, according to KDOW protocol. Multihabitat periphyton samples will be collected twice per year (critical period) for species identification. The in-stream substrate will be selected for sampling relative to its occurring abundance in order to accurately represent periphyton taxa from different habitat.

Dissolved Oxygen: Dissolved oxygen will be measured during every sampling event. During the low-flow summer period, 24 hour diurnal dissolved oxygen will be measured once at two select sites, one of which will be located at Clarks Run / KY52. The other site will be determined based on results of initial sampling.

Flow: Discharge, or flow, will be determined at all sites during each of the monthly site visits. Velocity and depth will be measured at intervals sufficient to characterize stream flow. Discharge will be computed as the sum of each velocity times the corresponding flow area. Pressure transducers are additionally mounted at six sites.

Physical Habitat Assessment: An EPA Rapid Bioassessment Protocol (RBP) worksheet will be completed at each site twice during the sampling year, once during the initial reconnaissance and once at the end of the year. Estimates of type, density, and aerial coverage of rooted aquatic plants (or lack thereof) will be determined by observation during monthly field visits. Physical channel condition will be characterized using Rosgen classification during this same period. For determining correlates for emergent plant and periphyton growth, canopy cover will be estimated using a spherical densitometer once during peak

leaf out and turbidity will be measured using a turdidimeter during periphyton (chlorophyll *a*) sampling.

1.4.4 Modeling

The TMDL modeling study of Clarks Run will address the following:

- Nutrients (nitrogen and phosphorus)
- Biochemical Oxygen Demand (as an indicator of organic enrichment)
- Dissolved Oxygen

The EPA model, Qual2K, will be used to predict pollutant concentrations based on environmental conditions during critical periods. Qual2K is a modernized version of Qual2E and is a one-dimensional steady state model.

Third Rock will deliver a TMDL document using the format outlined in the guidance document titled *Requirements for Kentucky DOW TMDL Documents*. This document includes descriptions of all relevant background information, summary, water body details, monitoring history, current monitoring effort, and modeling report. The steps required in creating this document are outlined below:

- Select modeling reach
    - Review existing in-stream data
        - Data will include all biological, chemical, and flow.
    - Find known point and nonpoint source pollutants.
        - Review land use mapping and aerials
        - Review available source loading data
        - Develop prediction tool for nonpoint source loading and relation to field data
- Segment reaches
    - Using land use cover and items above
- Select target time period (periods)
    - Review measured data, load data
    - Review all available flow data and precipitation records
    - Determine critical flow
- Set up Model Reaches
    - Input downstream point, lat/long, elevation (either USGS topographic or other available data)
    - Select velocity/depth computation method for each reach. Assign algae, SOD coverage coefficients.
        - Use Excel/VBA program named 'Shade.xls' or other estimate of daily shade factors
        - Review site photographs.
- Set up Model Climate: air temperature, dew point, wind speed (and height of measurement) and cloud cover
    - Find hourly data source close to project
    - Obtain data, format, QA/QC, input into model
    - Light and heat coefficients

- Point sources
    - Assign flow and chemical constituents (average of discharge monitoring report data, monthly operating data, or other)
    - Make assumptions about missing data, defend
    - Tributaries are not modeled explicitly but can be represented as point sources
- Non Point Sources
    - Assign flow and chemical constituents
- Select Rates: determine rates, constants, coefficients to use;
    - Calibrate model for spatial concentrations
    - Calibrate model for temporal dissolved oxygen concentrations
- Run sensitivity analyses for any parameters for which Third Rock does not have data and other parameters to determine model sensitivity
- Prepare modeling summary (estimate 20 pages)
- Select sensitivity scenarios for TMDL
    - Meet with KDOW to discuss load reductions
    - Run 10 scenarios
    - Summarize results

1.4.5 Training

After TMDL completion, Third Rock will provide continued support to KDOW with the further development of allocations, load reductions, and an implementation plan.

Two days of training regarding the model are anticipated with KDOW staff. This training will serve to describe the calibration of the model, the appropriate applications of the model, and the techniques for changing loads and parameters within the model. The training will include hands-on demonstration of the water quality model and creation of output tables and graphs. Training will also demonstrate how to apply the model to the anticipated, but not yet promulgated, nutrient criteria. This training will enable Division of Water staff to evaluate the effects of new nutrient criteria on load allocations.

1.4.6 Nonpoint Source Pollution Abatement

Practical solutions for known impairments will be recommended for the most significant pollutant sources. The feasibility of these solutions will be judged by cost, landowner cooperation, and long-term predicted success. Solutions will include on-the-ground best management practices, as well as potential funding options and the agencies responsible for implementing the funding.

1.5 Quality Assurance Objectives

1.5.1 General Quality Objectives

The overall project data quality objective (DQO) is to provide information that will lead to improved water quality and the removal of the tributaries upstream of Herrington Lake (and ultimately Herrington Lake) from the 303(d) list of impaired streams and reservoirs. Reaching this objective requires that data generated and used for modeling must be of sufficient quantity and quality to support:

- Determination of the source and extent of impairment to the tributaries of Herrington Lake.
- Development of a TMDL model for nutrients on Clarks Run by Third Rock.
- Development of a TMDL model for pathogens on Hanging Fork by KDOW

The following items detail the performance criteria for the measurement process associated with water quality sampling, water quality processing, and TMDL development for this project.

1.5.2 Field Objectives

Field observations and measurements provide data valuable for water quality assessment and modeling. Field sample collection directly affects the analytical results generated by the laboratories. The following specific tasks apply:

- Chain of Custody forms are to be completed such that custody of samples is traceable and accurate from the time of sampling until received by the laboratory.
- Samples are to be protected by proper packing and transportation, preservation and handling techniques in order to maintain the integrity of the sample.
- Cross-sectional measurements shall be sufficient to accurately characterize the flow area.
- Temporary markers and GPS positioning are established to ensure maximum repeatability in data collection position and to facilitate locating the sites by multiple parties.
- Field equipment will be calibrated in accordance with the manufacturer's instructions in order to meet the specified accuracy and precision criteria. Equipment calibration logs will be maintained.
- Grab collections are made to obtain samples chemically representative of the site during the time period and flow rate during which it is sampled.
- Total organic carbon shall be sampled with minimum headspace in order to minimize the impact of the volatilization of organic carbon.
- Habitat assessments are conducted in order to provide stream supporting capabilities, context to analytical assessments, record visual changes in the habitat and reference to measure remediation impact.

- EPA Rapid Bioassessment Protocol (RBP) are measured in order to provide a quantitative score of the waterbody indicating the quality of the environment.
- Photographs are taken to indicate and provide visualization for significant changes in the habitat throughout the duration of the sampling.
- Flow shall be measured with sufficient quality to determine the loadings of individual parameters at the time of collection.
- Periphyton and chlorophyll *a* sampling shall be conducted such that the surfaces sampled are representative of the site surfaces, algal speciation and growth levels.
- Passive high flow sampling shall be conducted such that the non-point nutrient runoff is captured at its peak.
- The pressure water level recorder measurements are used to establish more comprehensive flow measurements throughout the sampling period. These recorders are downloaded at a frequency to ensure all measurements are gathered.

1.5.3 Laboratory Analytical Objectives

The objective of the analytical parameters is to identify numeric or measurable indicators and target values that can be used to evaluate the TMDL and the restoration of water quality. Each parameter has a specific purpose that fits into this overall objective and shall meet the quality standards established in Table 2, Methods, Analytes, and Data Quality Indicators for the Dix River Watershed, and below.

- For modeling purposes, nutrient sampling will be conducted during varying flow events. The results of the nutrient samples will be used for modeling purposes and to rank and assess source pollutant levels. Nutrient sampling detection levels are similar to recent studies in the area (Lake Herrington study) and are adequate for modeling purposes.
- 15-day biochemical oxygen demand will be measured to determine the slow-acting oxygen demand, typically exerted by the nitrogenous components. It will be used as part of the oxygen balance of the stream and will indicate the downstream impact of oxygen demanding pollutant sources.
- 5-day carbonaceous biochemical oxygen demand will be measured to determine the short to moderated acting oxygen demand. It will also be used as part of the oxygen balance of the stream.
- Total suspended solids indicate a broad class of substances that may originate from natural or pollution sources. TSS may include phytoplankton, non-living particles containing nutrients and inorganic solids. As such, they affect the oxygen and nutrient balances (by mechanisms such as settling, recycling and light extinction).
- Total phosphorus will be measured to determine the phosphorus present in organic and inorganic forms. Phosphorus is a necessary nutrient for algae growth and contributes to eutrophication in Herrington Lake. It also affects the oxygen balance.
- Ortho phosphorus will be measured to determine the dissolved, inorganic phosphorus. This is the form most readily available for organism (algae) uptake. It is present in wastewater and is released during decay and recycling of particulate material.

- Nitrite as N is an intermediate product in both the nitrification and denitrification reactions that occur in natural waters. It is also a component of the total amount of nitrogen available, and as such affects algae growth and the oxygen balance.
- Nitrate as N is a form of nitrogen available for algae growth. As such it represents a pollutant contributing to eutrophication of Herrington Lake and impacts the oxygen balance. It is formed by the nitrification reaction in natural streams and is a pollutant found in agricultural runoff and wastewater.
- Ammonia as N is another form of nitrogen available for algae growth. It is present in sewage and agricultural runoff and affects the oxygen balance.
- Chloride is a conservative compound (*i.e.,* it does not react, settle or otherwise leave the water column) and may be used as a tracer for water flow. It contributes to specific conductance levels.
- Total Kjeldahl nitrogen is a measurement of the sum of total organic nitrogen plus ammonia. These forms of nitrogen represent nearly all the oxidizable nitrogen and therefore affect the oxygen balance of the stream.
- Total organic carbon measures living and dead organic matter, as well as indicating possible presence of herbicides and pesticides (which are generally organic compounds). Carbon is important for algae growth and organic particles can bind with nutrients and toxics.
- Alkalinity is the measure of the buffering capacity of the water, measured as calcium carbonate. Alkalinity is related to hardness, which affect metals' toxicity to fish.
- Total coliforms and *E. coli* samples will be collected to determine primary bacterial input locations. This sampling will be performed in Hanging Fork and Clarks Run to ensure that bacterial loadings are estimated for the bulk of the Dix River watershed. The analytical objective for both total coliform and *E. coli* is to establish a dilution series yielding real values for both analytes. To this end, the minimum detection limit is set at 1 MPN and the maximum as necessary to achieve real numbers. This dilution series will be continuously monitored and adjusted to achieve real numbers. For values reported as "greater than," modeling constraints will determine the proper use of the values.
- Chlorophyll *a* is an essential component of photosynthesis and is used as an indicator of phytoplankton concentration.
- Periphyton will be collected from natural substrate for two purposes:
    - First, monthly samples will be collected for chlorophyll *a* analysis. Results will be extrapolated to determine an algal biomass estimate as an indirect indicator of nutrient loading.
    - Second, because dominance of certain algal taxa can also indicate nutrient loading, multihabitat periphyton samples will be taken for species identification. The in-stream substrate will be collected relative to its occurring abundance in order to accurately represent periphyton taxa from different habitat.
- 24-hour Diurnal Dissolved Oxygen will be measured to examine the temporal dissolved oxygen dynamics. While algae (and other green plants) are photosynthesizing during the day, they produce oxygen. During the night, they respire and consume oxygen. Measuring the changes in oxygen demand over 24 hours will illustrate this and indicate

the amount of oxygen demand caused by photosynthetic organisms. (Note, temperature also influences the oxygen cycle and will also be measured during the 24-hour period.)

1.5.4 Data Quality Indicators

Data Quality Indicators (DQIs) are qualitative or quantitative descriptors of data quality. The quality of field and analytical data is most often assessed in terms of the DQIs including: Precision, bias, accuracy, representativeness, comparability, completeness, and sensitivity. A review of these indicators follows.

For laboratory data, the laboratory performs the initial review of the results and compares them with the DQIs. Cause analysis and corrective actions are taken if necessary and deviations from the DQIs are noted with appropriate data qualifiers. The Data Manager performs a secondary review of the data to assess the conformance of the laboratory data in conjunction with field quality controls to the DQIs.

For field data, the Data Manager provides the initial review of data quality, and additional review is provided as the data is compiled and evaluated by the modelers, et al.

1.5.4.1 Precision

Precision is the measure of agreement among repeated measurements of the same property under identical, or substantially similar conditions; calculated as either the range or as the standard deviation. Precision uncertainties will be measured through the collection of duplicate and split samples on 10 percent of collections that provide the overall measurement precision. The laboratory additionally performs duplicate samples with each analysis batch and is required to meet the requirements in Table 2, Methods, Analytes, and Data Quality Indicators for the Dix River Watershed. Subtracting the analytical precision from the overall precision provides the sampling precision.

The precision of RBP scores and general habitat assessment precision is controlled by the level of experience of the personnel conducting the assessment. Since the accuracy of the result is determined by the experience of the personnel recording the measurement, precision of results is also to be controlled by employment of high quality personnel. The initial and final RBP scores are assessed by personnel with a Master's degree and 5 years of experience in fieldwork. All personnel involved in assessment have been trained to properly conduct these assessments.

1.5.4.2 Bias

Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. Laboratories control bias by performing regular QC charting with which the acceptance windows for accuracy measurements are adjusted.

1.5.4.3 Accuracy

Accuracy is a measure of the overall agreement of a measurement to a known value; it includes a combination of random error (precision) and systematic error (bias) components of both sampling and analytical operations. Accuracy will be determined in the field through the use of spiked samples (10 percent of samples). For the laboratory, laboratory control samples (LCS) of known value and matrix spikes are used to measure accuracy according to Table 2.

1.5.4.4 Representativeness

Representativeness is a qualitative term that expresses the degree to which a portion accurately and precisely represents the whole. Representativeness in the field is achieved by adherence to applicable KDOW and EPA sampling methods. Homogenization of sample before analysis in the laboratory achieves representativeness. Samples are expected to be as representative as possible throughout the field and laboratory process.

1.5.4.5 Comparability

Comparability is a qualitative term that expresses the measure of confidence that one data set can be compared to another and can be combined for decisions to be made. Comparability of water chemistry results will be ensured through strict adherence to KDOW and EPA sampling and laboratory methods. Comparability of physio-chemical results will be ensured through regular probe calibration. Comparability of habitat data will be ensured through strict adherence to sampling protocols developed by the KDOW for in-stream habitat.

1.5.4.6 Completeness

Completeness is a measure of the amount of valid data needed to be obtained from a measurement system. It is expected that planned sampling will be 100 percent completed unless stream sites dry during summer months. Sites will not be relocated to avoid sampling overlap. A dry site will reflect zero nutrient and bacterial contribution of that section of the watershed.

1.5.4.7 Sensitivity

Sensitivity is the capability of a method or instrument to discriminate between measurement responses representing different levels of variable interest. Sensitivity for this project is achieved by adherence to the reporting limits listed in Table 2. Reporting limits are determined by a calculation based upon the method detection limit for analytical methods and instrumentation.

Sensitivity of sampling methods depends on the technique as well as the intent. The passive high-flow samplers will be constructed to simulate a grab sample but will be sensitive to the rate of water rise such that the analytical impact will be minimal.

1.6 Documentation and Records

1.6.1 General

In order to provide quality consulting to the KDOW, traceability and maintenance of documentation and records is essential. All records relating in any manner whatsoever to the project, or any designated portion thereof; which are in the possession of Third Rock shall be made available, upon request of the KDOW. Additionally, these records shall be available to any applicable regulatory authority and such authorities may review, inspect and copy these records. These records shall be retained for at least 3 years after the project is approved and closed by the EPA.

Third Rock will deliver a TMDL document using the format outlined in the guidance document titled *Requirements for Kentucky DOW TMDL Documents*. This document includes descriptions of all relevant background information, summary, water body details, monitoring history, current monitoring effort, and modeling report. Additionally, Third Rock will provide continued support to KDOW after TMDL Proposed Scope of Work completion with the further development of allocations, load reductions, and an implementation plan.

Third Rock will also deliver analytical data in a COMPASS format for all sampled stations. The number of stations and laboratory parameters for all project-monitoring stations is detailed on the attached spreadsheet. Hardcopy of data will also be presented to KDOW if requested. A specific list of the documentation to be included in the final report is listed below.

1.6.2 QAPP Management and Distribution

Key to these goals is the distribution of the most recent version of this QAPP to all parties listed on the distribution list once the QAPP has been reviewed and approved. The QA manager is responsible for ensuring that all applicable parties perform documented review of the QAPP. If, because of deviations in the QAPP, revisions are required, the QA manager shall ensure that all parties review the revised version. The current revision and the date of the revision shall be documented in the upper left hand corner of the QAPP pages. The QAPP shall be redistributed after all parties have reviewed the document.

1.6.3 Information Included in the Reporting Packages

A reporting package will consists of field data, chain-of-custody forms, and analytical laboratory reports. Specifically the final package will include copies of the following:

- Field observations recorded in the Sampling Technicians' field notebook
- EPA Rapid Bioassessment Protocol (RBP) worksheet (Figure 6)
- Data characterization and water quality datasheet (Figure 7)
- GPS Positioning and photographs
- Completed Chain-of-custody forms (Figure 8, uncompleted example)
- Analytical Laboratory Reports (Figure 9)

- Chlorophyll *a* Datasheets (Figure 10)

1.6.4 Data Reporting Package Format and Documentation Control

Data reporting packages will contain a consistent format and will be compiled initially during the quarterly meetings with KDOW and ultimately within the final report. Electronic data will be presented in Microsoft Word and/or Access (COMPASS format).

1.6.5 Data Reporting Package Archiving and Retrieval

The original copies of all field notes, field data sheets, lab sheets, chain-of-custody forms, and lab reports will be maintained and stored at Third Rock Consultants for the required document retention period for the grant. At the end of the required period, the documents will be archived in Third Rock's warehouse. Copies of all electronic data will be archived in specified Third Rock computer files. The laboratory shall also maintain all records associated with the analytical results including laboratory notebooks, bench sheets, instrument calibration and sequence logs, preparation logs, maintenance logs, etc. for the retention period of the grant.

2 Data Generation and Acquisition

2.1 Sampling

2.1.1 Sampling Process Design

The total area of the Dix River Watershed includes approximately 282,000 acres in central Kentucky and has been divided into several sub basins for the purposes of this project, as seen in Figure 3.

The lower Dix River Watershed includes the western edge of Garrard County, part of northern Lincoln County, and eastern portions of Boyle and Mercer Counties. The land is characterized by undulating terrain and moderate rates of both surface runoff and groundwater drainage. Most of the watershed lies above thick layers of easily dissolved limestone. Groundwater flows through channels in the limestone, so caves and springs are common in regions with this geology. Land use in the watershed is 90 percent agricultural and 5 percent residential. The surface waters of the watershed supply the drinking water for the municipal system in Danville. Businesses and organizations hold permits for discharges into the creeks. For the purposes of this project this watershed has been further divided into the Herrington Lake, Clarks Run, and Hanging Fork subwatersheds. Clarks Run and Hanging Fork are of particular concern for this project.

The lower Dix River watershed includes the river itself from the confluence with the Kentucky River near High Bridge to the mouth of Gilberts Creek southwest of Lancaster. Herrington Lake makes up much of this stretch of the Dix River. Among the creeks that feed the river within this watershed are Hawkins Branch, Boone Creek, White Oak Creek, McKecknie Creek, Tanyard Branch, Cane Run, and Rocky Fork. The watershed also receives water from the Dix River (upper), Logan Creek, Spears Creek, Mocks Branch, Hanging Fork Creek which drains approximately 18,000 acres, and Clarks Run which drains approximately 61,000 acres.

The assessed river segments in this watershed fully support their designated uses, based on biological and/or water-quality data. Herrington Lake does not support its designated uses, because of excess nutrient enrichment from a variety of sources. Phosphorus levels in the Dix River are elevated enough to cause potential nutrient enrichment problems (> 0.1 mg/L).

The upper Dix River watershed covers approximately 202,000 acres, in southern Garrard County, western Rockcastle County, and eastern Lincoln County. The land is characterized by undulating terrain, moderate to rapid surface runoff, and moderate rates of groundwater drainage. The watershed lies partly above fractured shales through which groundwater can easily move but which stores very little water.

The upper watershed of the Dix River includes the headwaters down to the mouth of Gilberts Creek just west of Gilbert (at US 27 between Lancaster and Stanford). Among the creeks that feed it are Negro Creek, Turkey Creek, Copper Creek, Fall Lick, Drakes Creek, Harmons Lick, Walnut Flat Creek, Cedar Creek, Stingy Creek, Turkey Creek, and Gilberts Creek. Land use in the Upper Dix watershed is

60 percent agricultural and almost 40 percent rural and wooded. Businesses and organizations hold permits for discharges into within this watershed.

In order to assess the load allocations for these areas, the following site types and as well as anticipated site visits are allocated as follows:

Watershed	Select Sites	Non-select Sites	Sampling Events
Clarks Run	4	4	96
Hanging Fork	6	8	168
Upper Dix River	1	7	96

The sampling and processing schedule is detailed in Table 1, on a monthly basis. From March 2006 to March 2007, monthly g*rab samples* will be taken at *all stream stations.* From November to April, *passive high flow sampling* will be conducted at the *select stations* with a goal of capturing one high-flow per month with a seven-day antecedent dry period. Because of the requirements to sample low, medium, and high flow events, the sampling events will be scheduled on a monthly basis by the Data Manager and Sampling Coordinator to maximize the potential of capturing these flow events. Scheduling of the sampling is on Third Rock's Work Schedule, which represents a comprehensive scheduling of all projects for which Third Rock is employed.

Site locations for the Dix River, Clarks Run, and Hanging Fork were chosen by Third Rock and GRW to specifically characterize the pollutant loadings and to facilitate modeling of these parameters in conjunction with dissolved oxygen. Spatial and temporal assumptions have specifically determined sampling location and the timing of sampling event. Stations will characterize pollutant contribution associated with specific sources of concern. Timing of sampling events will look at varying pollutant concentrations that could fluctuate with stream flow and volume. Samples will coincide will low, normal, and high flows. To determine nutrient loading associated with storm run-off, *passive high flow sampling* will be conducted at the *select stations* for all chemical parameters. Sampling periods will coincide with elevated storm-water flow with a goal of capturing one high-flow per month during that period that has a seven-day antecedent dry period though actual high flow sampling will be determined by rain intensity. Methods for passive high flow sampling will consist of a low-tech sampler.

During the elevated storm water flow, total coliform and *E. coli* will be sampled directly since the passive high flow sampling technique would bias the results. Technicians will be dispatched just prior to the storm to ensure the samples are collected during the elevated period.

2.1.1.1 Sampling Station Locations and Specifications

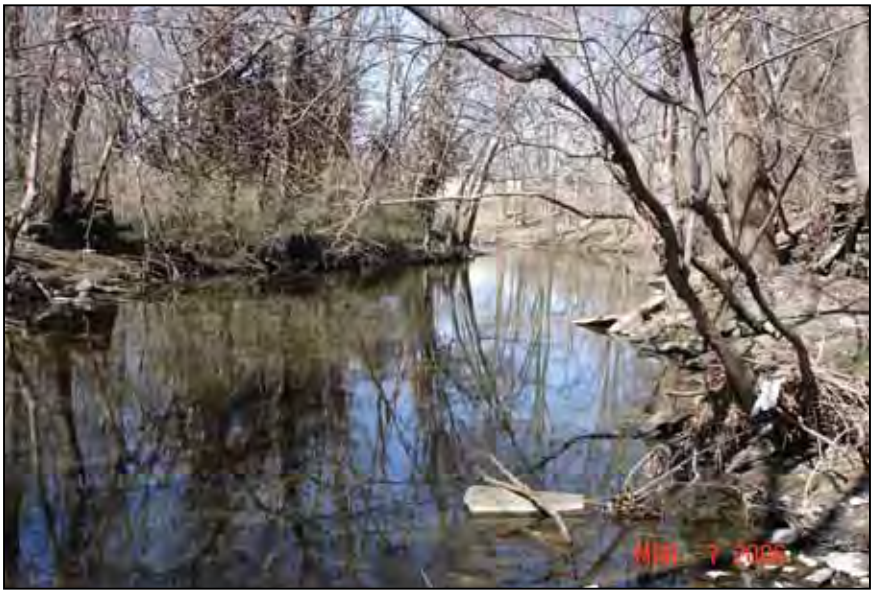
The specific criteria for site location are discussed below. Due to logistical constraints, stations are commonly located in close proximity to bridge crossings or culverts. Care is taken when locating stations so that sampling sites are far enough away from the bridges or culverts to minimize the influence of the inherent hydrologic modification caused by the anthropogenic modifications. A photograph of each sampling location (above each site) as well as the latitude and longitude (in that order) and a brief summary of the site conditions are included.

Clarks Run

Sites in the Clarks Run subwatershed have been located to discern nutrient and bacterial contributions from non-point sources (primarily cattle and residential), industrial facilities, potential sewage collection failures, and point-source contributions. The specific reasons for site selection are described below:

Corporate Drive- This non-select site is located in the headwater of Clarks Run. Based on land use, the location of this site corresponds primarily to NPS nutrient and bacterial contributions consisting primarily of agriculture with some residential sources. Located at 37.627177,-84.797265.

Clarks Run Bypass - Non-select site at the Danville US127 Bypass for characterizing potential nutrient and bacterial contribution from industrial and some residential sources. Located at 37.627177, -84.797265.

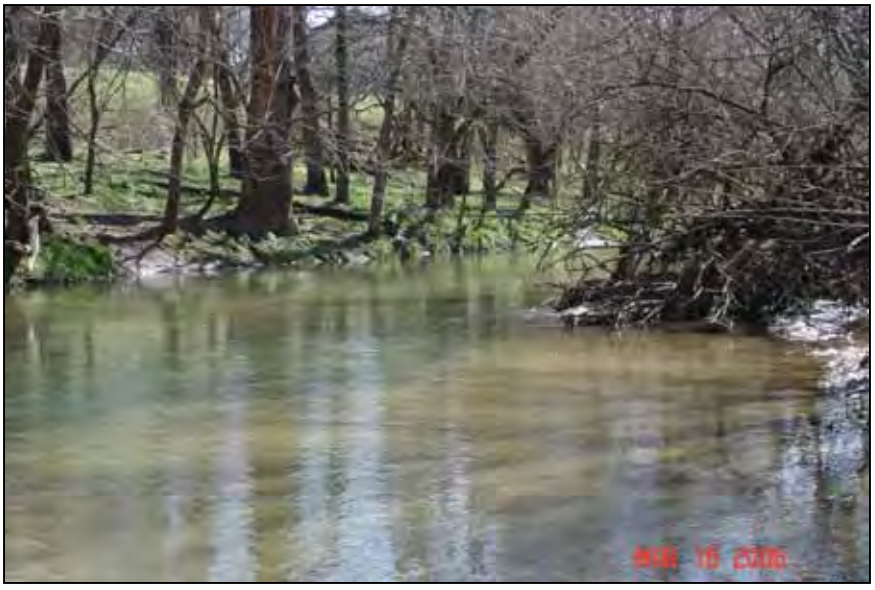


Second Street/Clarks Run – Select site to characterize the nutrient and bacterial levels directly attributed to a suspected sewage influx and before the WWTP outfall. This site is just downstream of Second Street. The extra storm-water sampling component of this select site will help insure an accurate representation of the pollutant loadings due to nonpoint source (NPS) and sewage contributions. Located at 37.635754,-84.772877.

<u>Clarks Run/KY52</u> – The primary select site, located above the KY52 bridge and above the confluence with Balls Branch, will assess the nutrient additions attributed to the Danville WWTP. Storm-water sampling at this select station will assess how nutrient concentrations from many sources vary with flow. Located at 37.631264, -84.735969.

<u>Clarks Run/Hwy 150</u> – Select Site to identify the nutrient and bacteria concentrations and potential industrial pollutants above the Danville WWTP. Storm water sampling could also discern the increased pollutant loads associated with heavy rainfall events. This site is located immediately downstream of a quarry discharge and just below the Highway 150 bridge. Located at 37.628470, -84.746087.

DOW Clarks - Select site at a historical DOW sampling location that will estimate the combined nutrient and bacterial contribution of Clarks Run and Balls Branch at all flow regimes. This site is just below Goggin Rd Bridge. Located at 37.638916, -84.721632.



Balls Branch Mouth- Select site to specifically characterize the NPS pollutant contribution from the entire Balls Branch watershed. Located at near the Balls Branch – Clarks Run confluence, 37.630455, -84.733358

Balls Branch West - Non-select site further up the watershed for pinpointing potential NPS contributions. Located at a Balls Branch bridge, 37.600947, -84.757055.

Hanging Fork

The Hanging Fork watershed is characterized primarily by agriculture (graze land) with a scattering of small communities having sanitary sewer outfalls. Stations are positioned to help pinpoint the location of major sources of nutrient and bacteria contribution from this watershed.

West Hustonville – Non-select site located in the upper reach of Hanging Fork. This station is positioned to estimate nutrient and bacterial loadings from headwater contributions upstream from Hustonville's WWTP outfall. Located at 37.470801, -84.821043

Baughman Creek - Non-select site located to estimate nutrient loading attributed to Baughman Creek watershed. This site is located immediately downstream of a school permitted discharge and before the Hustonville WWTP outfall. Located at 37.471207, -84.820744.

McKinney Branch - Non-select site located on a medium sized sub-watershed expected to have a significant NPS pollutant contribution. Located at 37.479748, -84.771170.

Chicken Bristle - Select site on the main stem of Hanging Fork located to characterize the nutrient and bacterial contributions of point and non-point sources and specifically the contributions from Hustonville's WWTP outfall. Located at 37.481364, -84.769010.

Frog Branch - Non-select site characterizing NPS loading in a distinct sub-watershed of Hanging Fork. Located at 37.505012, -84.758855.

Peyton Creek - Non-select characterizing NPS loading in a distinct sub-watershed. Located at 37.497558, -84.744313.

McCormick Church - Select site situated at this location for the purpose of estimating nutrient and bacterial loadings (point and non-point) from a group of several small drainages. Located at 37.526615, -84.742887.

Blue Lick - Non-select site located to estimate the agricultural NPS component of a medium sized drainage. Located at 37.527845, -84.731109.

Junction City - Non-select site that drains a residential/agricultural area west of Junction City. Located at 37.566007, -84.806433.

<u>Oak Creek</u> - This select site will catch the urban runoff (and outfall) from the majority of Junction City as well as an agricultural drainage. Located at 37.558674, -84.790585.

<u>Moores Lane</u> - Non-select site to determine specific sub-watershed contribution of Harris Creek. Located at 37.544012, -84.781899.

Knob Lick Creek - Select site will catch some additional drainage from Junction City plus the accumulation of potential pollutants from all the sites above. Located at 37.551944, -84.730426.

Hanging Fork/Hwy 150 - Non-select site located here to estimate the accumulation of potential pollutants near the convergence of two large subwatersheds. Located at 37.573390, -84.700117.

<u>Hanging Fork Mouth</u> - Select site located to estimate the total loading of nutrients and bacteria attributed to the Hanging Fork watershed. Located at 37.623639, -84.680562.

Upper Dix River

The sites in this section of the watershed are located upstream of the Hanging Fork confluence with the Dix River. Similar to the Hanging Fork subwatershed, this area contains primarily agricultural grazed with rural residences and small communities (with WWTP outfalls). Though the data from these sites will not specifically be used for TMDL calculation, the resultant information will help determine and rank the significance of nutrient, TSS, and bacteria contribution of this drainage to Herrington Lake.

<u>Gum Sulfur</u> – This non-select station was located to account for the nutrient contribution of a WWTP outfall at Brodhead. Located at 37.427359, -84.452234.

Copper Creek - This non-select station was located at the mouth of Copper Creek to account for NPS runoff from a significant subwatershed with an abundance of cattle. The stream section immediately upstream of the site is listed as partially supporting for aquatic life. Located at 37.455167, -84.471822.

Crab Orchard – This non-select station was located to account for a Dix River WW outfall from the community of Crab Orchard. Due to lack of access, station could not be located directly below outfall. The first available sampling location was determined to be the KY 39 bridge because of braided channel issues directly upstream. Located 37.490419, -84.512426.

<u>Drakes Creek</u> - This non-select site encompasses two large drainages with an abundance of cattle (Drakes and Harmons Creeks). Located at 37.504822, -84.518456.

<u>Gilberts Creek</u> - Site was located to catch the pollutant contribution of the Gilberts Creek drainage (primarily NPS) and also an unnamed tributary with a point-source (KPDES storm water discharge) that carries urban runoff for the city of Lancaster. Located at 37.571167. -84.596938.

White Oak - Located directly below Lancaster's W WTP outfall. Data from this site will characterize nutrients and bacteria level contributions from the facility. Located at 37.605136, -84.592481.

Dix above HF - This select station will measure the NPS nutrient runoff associated with the Dix River above Hanging Fork. Located at 37.602466, -84.634587.

Dix DOW (below HF) - Non-select site at a historic DOW location. Data from this site will estimate the pollutant loads from the combination Dix and Hanging Fork. Located at 37.640959, -84.662930.

2.1.1.2 Inaccessibility Contingency Planning

If sample sites must be relocated due to unseen issues, the site will be relocated to best suit the desired goal of the project. New sites will be given new names and IDs to maintain consistency of results.

If samples cannot be collected at a station due to dry conditions, the station will not be relocated. The effective loading of pollutants will be zero and modeled as such. If a site cannot be reached during the specified sampling period, a re-sampling event will be scheduled as soon as possible to best estimate the conditions at the time of the specified sampling period.

2.1.1.3 Critical vs. Non-Critical Parameters

Critical Parameters are those parameters that are absolutely necessary for the completion of the project. The high-flow samples from select stations (using passive high flow samplers) will be designated as "critical" due to the importance in timing the collection and retrieval of the water sample.

Because they are directly tied to the objectives of the study, the following parameter are also considered critical:

- Biochemical Oxygen Demand, 5-Day Carbonaceous
- Phosphorus, Total and Ortho
- Nitrate as N

- Ammonia as N
- Total Kjeldahl Nitrogen
- Total coliforms and *E. coli*
- Chlorophyll *a*
- Physiochemical Measurements
- Habitat, at least once
- Photographs, at least once
- Flow

All other parameters are either supplemental or could be estimated (derived) from the other measurements based on previous monitoring or typical surface water interactions and are therefore designated as non-critical.

2.1.1.4 Sources of Variability

Sources of variability associated with field sampling are inherent and often unquantifiable. For example, environmental conditions associated with climate (e.g., microhabitat fluctuations in temperature, rainfall, etc. between stations) and flow (e.g., timing of samples in regards to measuring the transport of pollutants in an identical water mass as it travels downstream) are typical forms of variability in a field sampling project of this type and often cannot feasibly be accounted for. The variability associated with environmental conditions in this project will be lessened to a degree by the efficient timing of sample collection during specific weather conditions and flow regimes. Using three teams for data collection will reduce temporal variation in samples.

In the field, variability associated with equipment is primarily limited to the water quality probes and measuring devices. Variability associated with these devices can be found in Table 2. The Hydrolab DS5 multi-probe is equipped with four primary sensors, pH, dissolved oxygen, conductivity, and temperature. Turbidity may also be measured on the Hydrolab or by turbidimeter. The velocity current meter may fitted with two propellers depending on the depth and the amount of flow present. The smaller propeller requires less depth to measure the velocity but is less sensitive. Variance in flow measurements may additionally be compounded by objects in the stream which impede flow (i.e. algal growth) or by the number of points sampled across the flow area.

To reduce the variability associated with flow measurements made by velocity meter, several procedures are conducted. To increase accuracy in streams with large variables in depth or velocity, measurement intervals are reduced from 3 ft to sizes that better characterize the entire cross-section. The first and last velocities are also measured closer to the banks to reduce error. Because water velocities may change at larger depths, streams deeper than 2.5 ft are measured at two depths. Algal growth that may interfere with the proper functioning of the propeller of the velocity current meter is scraped away from the location of the measurement to reduce this variability. Repeating the float technique three times reduces variability in simple float estimation of velocity.

In addition to field equipment, the Rapid Bioassessment Protocol (RBP) worksheets can be a source of potential variability during physical stream assessment. The intrinsic subjectivity of the physical habitat scoring using the EPA RBP method is a concern for the Dix River Watershed project. To ensure

consistency and accuracy with this assessment, Third Rock staff undergoes yearly in-house training that strictly pertains to the EPA RBP scoring protocol. Training methods are based on tutorials provided first-hand to Third Rock by U.S. Army Corps of Engineers (Louisville District). In addition to this training, sampling stations on the Dix River project RBP sheets are also consistently filled out by the same experienced biologist at all sites. Assessments are performed by personnel with a Master's degree and 5 years of experience in fieldwork.

Variability in regards to water sample collection will be minimized by a strict adherence to collection protocols. Consistent field personnel will also reduce variability associated with collection.

2.1.2 Sampling Methods

During all sampling activities, sampling methods and gear will utilized is analogous to EPA and KDOW recommendations. Specific methods are detailed in the following sections. All samples are to be collected in bottles according to the analytical methods referenced in Table 3, Summary of Project Sampling and Analytical Requirements.

2.1.2.1 Grab Sample Collection

Samples shall be collected directly from the source. When collecting samples, latex gloves shall be used to prevent contamination. The sampling technician will collect the sample by submersing a decontaminated rinsed stainless-steel bucket into source as to obtain a representative aliquot. Submersion shall only be to the bucket mid-depth, taking caution not to scrape the bottom of the source minimizing excess solids. An appropriate sized bucket relative to the bottle(s) being collected shall be used. The bucket size should be sufficient to completely fill the sample bottle(s) from a single submersion. Take care to avoid overfilling in bottles containing preservative. Fill pre-labeled collection bottle(s), per method specifications, directly from the bucket.

Stream samples will be collected from the thalweg (or low water channel) just above the stream bottom. Bottles will be filled to near 100 percent capacity. Efforts will be made not to stir up sediments during collection. Proper field data sheets will be completed. Samples will be labeled accordingly, placed on ice, and delivered to CT Laboratories Laboratory within the required holding time(s). Proper chain-of-custody procedures will be followed to ensure accuracy in sample reporting. Field quality controls, as specified in Section 2.3: Quality Control will be collected at this time.

Care will be taken when filling total organic carbon (TOC) sample bottles to avoid unnecessary agitation of water and to ensure complete filling of bottle, as headspace in the bottle will cause bias of results due to volatilization of organic carbon.

2.1.2.2 On-site Assessment

During initial setup of the site locations, several tasks were completed at each station:

- Permanent monuments (survey pins) were established to standardize water collection, flow measurement, and photograph locations at each station.

- Passive high flow storm-water sampling device locations were determined and installed (select stations only).
- Cross-sectional measurements were completed at each station to support discharge computation. For each cross-section, three reference points were established. Two of the points, located on opposite sides of the bank, were located for subsequent section measurements. The third point was located for reference of stage (tape-down) readings. Stage reference points may be located on a bridge, established with pins (rebar), or a sturdy overhanging limb.

This work was done to aid in the measurements as listed below:

2.1.2.2.1 Habitat

During habitat assessment, at the initial and final station visits, a thirty-minute visual inspection will be completed at each stream sampling station or reach. Ten habitat parameters will be assessed, according to Methods of Assessing Biological Integrity of Surface Waters in Kentucky (KDOW 2002), including epifaunal substrate (quantity and variety of substrate), embeddedness and pool substrate characterization (measurement of silt accumulation and type and condition of bottom substrate, respectively), velocity/depth regime & pool variability (combination of slow-deep, slow-shallow, fast-deep, and fast-shallow habitats and measurement of the mixture of pool types, respectively), sediment deposition (accumulation in pools), channel flow status (the degree that the channel is filled with water), channel alteration (measurement of large-scale changes in the shape of the channel), frequency of riffles & channel sinuosity (sequence of riffles and meandering of the stream, respectively), bank stability (measure of erosion), bank vegetation (amount of vegetative protection), and riparian vegetative zone width (width of the natural vegetation from the edge of the stream bank through the riparian zone). All of these criteria are rated (1 to 10) and combined to obtain a habitat score (0 to 200) that can be compared to a reference condition. Use attainment can be estimated based on the habitat score.

Once during the period of peak leaf out, the canopy cover will be estimated using a spherical densitometer. To use the spherical densitometer, the instrument is held level, 12 to 18 inches in front of the body and at elbow height so that the Sample Technicians head is just outside of the grid area. Each square on the grid is divided in four and systematically counted for canopy openings. The total count is multiplied by 1.04 to obtain a percent of the overhead area NOT occupied by canopy. The difference between this number and 100 provides the estimated percent canopy coverage. Four readings shall be recorded and averaged while facing north, south, east, and west.

2.1.2.2.2 Flow

In order to determine stream discharge or flow (Q), measure the flow area (A) and water velocity (V). Flow is calculated according to the following equation for increments across the stream.

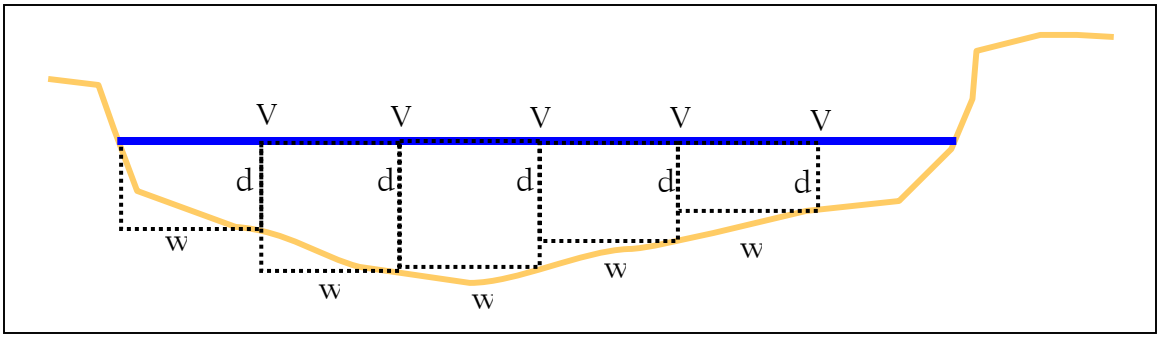
$$Q = V * A$$

where:
Q = Discharge or Flow (ft^3/sec)
V = Velocity (ft/sec)
A = Flow Area (ft^2)

In order to measure the flow area, three methods are used. For all stations, a stream cross section is surveyed (via Total Station). For six select stations, this information can be used in conjunction with a pressure transducer water level recorder (Infinities USA) to determine the flow area. If the water level is measured at the cross-section with a staff gauge or marked with pins on the stream bank, the flow area can also be calculated. Alternatively, the stream may be waded at the cross-section to determine depth and breadth at the time of the sampling visit. Velocity can be measured by a current meter or a floating object.

On a monthly basis, the flow for all streams low enough to wade will be measured according to USGS 2000. Velocity and water depth are measured at intervals across the stream sufficient to characterize discharge. A 100-ft tape is stretched across the stream in the established cross-section to indicate the intervals. Typically, stream depth and velocity are measured at 3 ft intervals across the stream. The interval is adjusted as necessary to thoroughly characterize the entire cross-section of flow. Points should be closer together if there is a lot of variation in the depth or velocity of the cross-section. Notes are made during the data collection to indicate any special conditions observed.

The approximate area of each flow box is the depth of water at a given point multiplied by the width of the flow box. This concept is illustrated in the figure below. The convention for calculating flow is to apply a measured velocity and stream depth to the width between that station and the previous station. To increase the accuracy of flow calculation, the first and last velocity and depth measurements should be made as close to the banks as is feasible.



Stream cross-section showing intervals where water depth and velocity are measured. Flow will be calculated for each "box" (flow area for each box is d * w) and summed to obtain the flow for the entire stream.

At each station within the cross-section, velocity is measured with a General Oceanic current meter mounted on a rod, where velocity is indicated by the number of revolutions of the propeller over a given time interval. The individual using the velocity meter should hold the rod vertically in the profile with the meter parallel to the direction of stream flow and stand at least 1 ft downstream and to the side of the velocity meter so as not to interfere with the current. Velocity is measured for approximately 60 seconds.

Average velocity is measured at 0.6 of total stream depth when the depth is less than 2.5 ft. When the stream is deeper than 2.5 ft, velocity is measured at 0.2 and 0.8 of the total depth and the average of the two readings is used as the average velocity at that point for discharge calculations. Discharge (Q) is

calculated for each interval of the stream where velocity and depth are measured and total stream discharge is calculated as the summation of the discharge from each interval. Water depth is also recorded at a single known point in the stream during each visit.

When the stream is too deep to wade with the current meter, stream velocity is roughly estimated using a floating object. The float can be any buoyant object, such a partially filled plastic water bottle. Ideally, it needs to be heavy enough so that about an inch of it is below the water line. When the floating object cannot be retrieved from the stream, a "weighty" yet compact piece of stick/wood is used. When feasible, a 50 ft section of stream is measured for the float test. The float is released out into the stream in a location most representative of the entire stream and the time is recorded for it to travel the known distance. If the float moves too fast for accurate measurement, a longer travel distance will be measured. The simple float estimation of velocity will be repeated for a total of three trials. The surface velocity values obtained by this method are corrected to represent mid-depth velocity (Daugherty *et al*. 1985).

$$mid-depth\ stream\ velocity = 0.8 \times surface\ velocity$$

Discharge during high flow is estimated using this velocity measurement, cross-section information, and depth measured from the pressure transducer water level recorder, staff gauge, or pins on the bank.

At stream velocities below the measurable range of the current meter, the propeller will not turn. If the stream velocity is too low to be accurately measured by the current meter, it may be necessary to estimate stream velocity using the simple float. If the velocity is below the limit of the current meter, the stream will still be waded and water depth will be recorded at intervals across the stream. The velocities obtained by the float test (three trials) during low flow conditions will be compared to the known lower limit of the meter.

2.1.2.2.3 Physio-chemical measurements

Temperature, dissolved oxygen, conductivity, and pH will be measured during field sampling of the streams with a Hydrolab water quality instrument. Operation of the Hydrolab instrument is conducted in conformance to the Hydrolab operation manual (Hydrolab, 1997).

During the low-flow summer period, 24 hour diurnal dissolved oxygen will be measured with the Hydrolab once at two select sites, one of which will be located at Clarks Run / KY52. The other site will be determined based on results of initial sampling. The Hydrolab will be deployed for a 24-hour period during which its data-logging feature will store the dissolved oxygen data.

Global Positioning System coordinates will be obtained using a Garmin GPS or the equivalent, accurate to ±5-40m. Readings are measured in NAD83. Internal SOPs and manufacturer's instructions will be followed to record these measurements.

2.1.2.3 Periphyton Sampling

Periphyton sampling is to be done in accordance with the *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (KDOW 2002). To meet these objectives, the Sampling Logistics Coordinator built a Periphyton Substrate Vacuum. Based on KDOW 2002 methods, this vacuum consists of a 3-inch diameter PVC pipe used in conjunction with a neoprene rubber gasket attached to a hand operated pump. To sample periphyton from stations, the gasket end of the PVC is pressed against the bedrock substrate so that the periphyton within the area enclosed can be dislodged with a stiff bristle brush. The hand operated pump is then inserted into the PVC pipe (still being pressed against the bedrock) and the periphyton is pumped into a filer flask using the hand operated pump. Five replicates are taken for a total area of 0.25m^2. This portion is sent to the laboratory for analysis by a modified version of Douglas 1958.

2.1.2.4 Chlorophyll *a*

Chlorophyll *a* samples will be filtered in Third Rock's lab before transporting to CT Laboratories for analysis. Initially, the time, date, and volume of the sample will be recorded on a Third Rock bench sheet (Figure 10). A measured volume of water from each sample will be filtered through 0.45µm cellulose membrane filters. For each sample, water will be filtered and particulate matter will be collected on three membrane filters, folded in half and enclosed within aluminum foil. Each sample will then be placed in a zip-lock bag, labeled with the filtered volume of water, and frozen before delivery to the lab. The bench sheet will accompany the filtered sample with the information regarding date/time of collection, date/time of filtration, volume of filtered sample and area of aspiration.

2.1.2.5 Passive High Flow Sampling

Sampling periods will include an elevated storm flow between November and April with a goal of capturing one high flow per month during that period with a seven-day antecedent dry period. Methods for passive high flow sampling will consist of a low-tech sampler based on methods presented in Subcommittee on Sedimentation, 1961. Sample bottles are mounted on an in-stream frame. Bottles fill with water as the stream rises. Once the bottles fill, samples will be collected for analysis. Technicians will frequently observe the sites when conditions are optimum for filling the bottles from the high flow.

2.1.2.6 Pressure Transducer Water Level Recorder

At 6 of the 11 select locations, stream water level is continuously monitored using a pressure water level recorder (Infinities, USA). These sites include Drakes Creek, Dix Above, Knob Lick, Hanging Fork 150, Clarks Run Bypass, and Balls Branch Mouth. The pressure sensor measures water depth and digitally records the data on a user defined interval. For this project, the device records water level readings every 20 minutes. The pressure sensor is accurate to +/- 0.1 percent of the measurement range and the resolution is 0.01 inches.

2.1.2.7 Sampling Equipment

For the purposes of this project, the following equipment will be utilized in the sampling effort:

- Periphyton Substrate Vacuum
- Filtration Apparatus
- Hydrolab MS5 and associated probes
- Rising stage passive high flow sampling apparatus
- Infinities USA continuous pressure transducer water level recorder
- General Oceanic current meter
- Garmin GPS
- Turbidimeter
- Spherical Densiometer

2.1.2.8 Decontamination and Sample Integrity

During all sampling events, precautions will be taken to ensure the integrity of the collected sample. These tasks include:

- Labeling sample bottles with time and date before filling with water to ensure ink legibility.
- Traceable custody shall be documented from the time of sampling until delivered to the laboratory.
- Wearing latex gloves during all sampling events to avoid potential sample contamination.
- Rinsing sampling equipment between sites with deionized water
- Avoidance of streambed sediment agitation during sample collection
- Immediate placement of sample bottles in ice-filled coolers
- Wrapping chlorophyll *a* bottles in aluminum foil (until filtered) to block light penetration
- Prompt delivery to laboratory for analysis

Cleaning and decontamination of the sampling equipment includes:

- For standard collection parameters, the stainless steel collection bucket will be rinsed three times with site stream water.
- The Hydrolab is to be rinsed with soapy water and rinsed with D.I. water daily. The instrument is to be rinsed with D.I. water between use at each sampling site.
- All rinsate is to be disposed of into the watershed, downstream of the sampling site, as the constituents do not represent a threat to the watershed area.

2.1.2.9 Problems and Corrective Action

Known or suspected deviations from sampling methods, the protocols of this QAPP, or other applicable protocols are to be reported to the Project Administrator. These incidents are documented by email to the project folder and the Project Administrator. All project related emails are to be sent to a central project electronic folder for recall and storage. If the deviation represents a serious flaw with sampling

methodology, sampling results, or modeling methods, corrective action will be taken based on recommendations the project administrator receives from the KDOW.

2.1.3 Sample Handling and Custody

2.1.3.1 Chain-of-Custody

Chain-of-custody (COC) forms will be completed for all samples collected in the field and will follow each sample throughout sample processing. A Chain-of-Custody form is a controlled document used to record sample information and ensure the traceability of sample handling and possession is maintained from the time of collection through analysis and final disposition. A sample is considered in custody if it is:

- In the individual's physical possession,
- In the individual's sight,
- Secured in a tamper-proof way by that individual, or secured in an area restricted to authorized personnel.

The Data Manager and Sampling Coordinator shall create COCs and provide to the Sampling Technicians. All information shall be documented on the COC in black or blue waterproof permanent ink including field physio-chemical measurements and custody information.

The Sampling Technician shall initiate sample custody at the time the sample is collected. Field custody documentation shall include:

- Verification of Sample Identification
- Number of Sample Bottles Collected
- Collection Date
- Collection Time
- Collector's Signature

The Sampling Technician shall maintain possession of the sample until custody is transferred to the laboratory or another party. The COC shall accompany the sample from the time of collection until it is relinquished. Field custody is relinquished by signature, with date and time, of the Sampling Technician in the designated area on the COC.

2.1.3.2 Sample Handling and Transport

The Sampling Technician is responsible to ensure that lids to all bottles are secured properly and tight to prevent leakage. All samples shall be collected and preserved as specified in Table 3, Summary of Project Sampling and Analytical Requirements. Glass bottles are placed in appropriate bubble wrap material to protect against breakage during shipment.

Sample bottles are placed in coolers lid side up. Samples are transported according to method storage requirements. Samples requiring storage at 4 ± 2°C are placed inside plastic bags to ensure that sample labels stay dry during transport. The bagged samples are placed in an appropriately sized cooler in

order best pack the samples with an adequate amount of ice, ensuring the appropriate temperature is maintained until arrival at the laboratory. Additionally, loose ice is placed around the bagged samples.

Samples coolers should be of adequate size to allow ice to surround all sample bottles. It is the responsibility of the Sampling Technician to ensure that coolers are properly packed and that they have sufficient cooler space on their vehicle for their daily sample load. Coolers shall be secured during transport such that significant disturbance of the samples is avoided.

Upon receipt at the laboratory, the sample custodian shall review the COC for completeness and accuracy. Anomalies shall be documented. The laboratory shall measure sample temperature upon receipt; determine if sample aliquots have been placed in appropriate bottles and properly preserved, by verification with pH strips, as applicable; findings shall be documented on COC, and inspect the sample for proper identification and bottle integrity; any discrepancies and/or bottle damage shall be documented on the COC.

2.1.3.3 Sample Labeling and Identification

Empty samples bottles are shipped from the analytical laboratory with preprinted information to assist in the proper identification of samples. These labels indicate Third Rock's name and project identification, and the expected parameters to be analyzed from that bottle. Sampling Technicians are responsible for recording the sampling station, which serves as the sample identifier, as well as the date and time of the collection on each sample bottle as well as on the COC. In the event that a preprinted label could not be obtained from the laboratory, the Sampling Technician would be responsible for recording the information listed on these labels on the sample. If possible, apply labels before sampling as moisture on the sampling bottles can make adhesion of the label to the bottle difficult.

2.2 Analytical Procedures

Water samples will be analyzed for several parameters following standard methodology as listed in Table 3. Modifications to the prescribed and/or pre-approved analytical methods will not be made without the knowledge and consent of Third Rock's Project Administrator.

As current regulations do not specify specific target limits for the analytes involved, the laboratories regular reporting limits were cited for this project. The reporting limits of the analytical laboratory are recorded in Table 2, along with other performance criteria, and are for analyses of samples within the calibration ranges for the individual methods. The reporting limits of individual sample may be raised if a dilution is required to quantify the target compound(s) within the acceptance range.

Since dissolved oxygen is of special concern for this project, three types of analyses for biochemical oxygen demand were selected. BOD-5 is the standard analysis of biochemical oxygen demand over a period of 5 days. BOD-15 is a modification of the BOD-5 in which the samples are allowed to incubate for a period of 15 days.

In order to properly analyze the parameters associated with the project, the laboratory is required to calibrate and maintain instrumentation and equipment. A list of the key equipment / instrumentation includes:

- Spectrophotometer
- Inorganic Flow or Discrete Autoanalyzer
- Ion Chromatograph
- Air Incubator
- Carbon Elemental Analyzer
- Dissolved Oxygen Meter

2.2.1 Problem Resolution and Corrective Action

The laboratory is required to maintain a corrective action and cause analysis system in order to address deviations and client complaints. When a deviation from an internal procedure or external method or protocol is found or a client has a complaint about the data results or service, the laboratory shall document these incidents and begin a cause analysis to determine the source or sources of the problem. Once the source(s) is (are) identified, the laboratory shall institute corrective action to achieve compliance. Evidence of completion of this corrective action and follow up evaluation of the effectiveness of the action, as necessary shall demonstrate compliance.

2.2.2 Sample Disposal Procedures

In general, samples are disposed of 30 days after results have been reported to the client. All sample bottle labels are removed or obliterated prior to disposal.

Hazardous wastes are returned to the client for disposal. The lab maintains status as a limited quantity generator of hazardous waste. As such, other hazardous solid wastes are disposed of in a hazardous waste designated dumpster and sent directly to an in state permitted landfill.

Non-hazardous aqueous samples are disposed of by pouring the neutralized sample into a conventional drain to the municipal sewage treatment system. Non-hazardous solid wastes (including emptied bottles from aqueous samples) are disposed of by placing in a dumpster for municipal landfill disposal.

2.2.3 Turn around Times

It is the expectation of Third Rock Consultants that laboratory analyses are completed before the next scheduled sampling event, where possible.

2.3 Quality Control

Chemical data quality will be ensured through strict adherence to KDOW (2002b, 1995). Approximately 10 percent of water samples will be duplicated or split and sent to CT Laboratories for analysis.

- Field Duplicate Sample

Approximately five percent of all samples taken in the field are duplicated. To perform a field duplicate, the Sampling Technician shall consecutively collect two representative aliquots, independent of one another, from the same source by the grab collection technique.

- Field Split Sample

Approximately five percent of all samples taken in the field are split. To perform a field split sample, the Sampling Technician shall evenly divide the contents of one grab collection into two sets of sampling bottles. To ensure the split is representative, sample bottles are each filled in three rounds of filling each bottle one third of the total volume.

To ensure that data of known and documented quality are generated in the laboratory , the QC criteria described in this section must be met for all analyses, as applicable. The Laboratory QA Director is responsible for monitoring and documenting procedure performance, including the analysis of control samples, blanks, matrix spikes, and duplicates.

- Blanks

A method blank (MB) is prepared at a frequency of one per 20 field samples depending on the specific method. The MB is analyzed at the beginning of every analytical run and prior to the analysis of any samples. MB results are acceptable if the concentrations of the target analyte does not exceed the reporting limit (RL). If any target analyte concentration in the MB exceeds the RL, the source of contamination must be identified and eliminated. Analysis of samples cannot proceed until a compliant MB is obtained.

- Duplicates

A duplicate sample (DUP) or duplicate matrix spike sample (MSD) is prepared at a frequency of one per 20 field samples depending on the specific method. The relative percent difference (RPD) between duplicate samples, for samples having analyte concentrations greater than their respective reporting limit, or between a matrix spike (MS) and matrix spike duplicate (MSD), must be within the acceptance ranges. If the QC criteria for duplicate sample or spike analyses are not satisfied, the cause of the problem must be determined and corrected. If the problem adversely affected the entire analysis batch, all samples in the batch must be reanalyzed.

- Matrix Spikes

Spikes (MS) are prepared every 20 field samples for each matrix, depending on the specific method. Spike recoveries must fall within the acceptance ranges. If the QC criteria for the matrix spike analyses are not satisfied, the cause of the problem must be determined and corrected. If the problem adversely affected the entire analysis batch, all samples in the batch must be reanalyzed.

- Laboratory Control Samples

A laboratory control sample (LCS) is second-source to the calibration standards and must be prepared at a frequency of one per every 20 field samples depending on the specific method requirements. The LCS results are acceptable if the percent recovery of each analyte is within the determined acceptance

range. If the LCS results do not meet specification, sample analyses must be stopped until the problem is corrected, and all associated samples in the analysis batch must then be reanalyzed.

2.3.1 Calculations

The following calculations are used in the interpretation of the data provided by the quality controls:

- Accuracy

For LCSs, calibration standards or additional QC samples of known concentration, accuracy is quantified by calculating the *percent recovery* (%R) of analyte from a known quantity of analyte as follows:

$$\%R = \frac{V_m}{V_t} \times 100$$

where:

V_m = measured value (concentration determined by analysis)
V_t = true value (concentration or quantity as calculated or certified by the manufacturer)

A matrix spike (MS) sample or a matrix spike duplicate (MSD) sample is designed to provide information about the effect of the sample matrix on the digestion and measurement methodology. A known amount of the analyte of interest is added to a sample prior to sample preparation and instrumental analysis. To assess the effect of sample matrix on accuracy, the %R for the analyte of interest in the spiked sample is calculated as follows:

$$\%\,R = \frac{(SSR - SR)}{SA} \times 100$$

where:
SSR = spiked sample result
SR = sample result
SA = spike added

- Precision

When calculated for duplicate sample analyses, precision is expressed as the *relative percent difference* (RPD), which is calculated as:

$$RPD\,(\%) = \frac{|S - D|}{(S + D)/2} \times 100$$

where:
S = first sample value (original result)
D = second sample value (duplicate result)

2.4 Instrument / Equipment Maintenance and Calibration

All sampling equipment will be maintained and calibrated according to manufacturer recommendation.

The Hydrolab runs on battery power and thus the charge must be maintained by charging on a daily basis. Calibration shall be completed in accordance with the user manual (Hydrolab, 1997) on a weekly basis.

All supplies are acquired through Third Rock Consultants' vendors. The members on this vendor list have applied quality control measures that have resulted in recurring quality.

All maintenance on laboratory equipment is conducted in accordance with manufacturers' recommendations. These requirements are described in the laboratories' standard operating procedures and appropriate instrument maintenance manuals. The applicable laboratory is responsible for ensuring that timely maintenance is conducted and that sufficient spare parts are on hand for necessary maintenance and repair procedures.

The frequency of maintenance performed depends on the equipment; laboratory maintenance is scheduled and conducted daily, monthly, weekly, quarterly, semiannually, and annually, as required. A few maintenance needs (e.g., accidental breakage, part failure) are not covered by the general maintenance schedule, and such maintenance is performed as needed.

Specific instrument calibration requirements can and do vary slightly depending on the particular method and the project and regulatory requirements for the project. Detailed descriptions of specific calibration requirements are provided in the laboratory analytical method SOP for each method.

2.5 Non-Direct Measurements

Non-direct measurements include any measurements or data that will be used during this project that will not be directly measured by Third Rock or its subcontracted partners.

The EPA model, Qual2K, will be used to predict pollutant concentrations based on environmental conditions during critical periods. Qual2K is a modernized version of Qual2E and is a one-dimensional steady state model. When modeling, weather data will be obtained from a third party source, such as the National Climatic Data Center. Also pollutant source assessment relies on non-direct measures (i.e. land use, watershed characterization) when modeling loads from nonpoint sources.

2.6 Data Management

Records are to be stored until 3 years after the close of the project. An efficient and effective data management system is necessary to maintain and store all project related data.

The laboratory is expected to maintain all records associated with the analytical results; including laboratory notebooks, bench sheets, instrument calibration and sequence logs, preparation logs,

maintenance logs, etc.; for the retention period of the grant according to their internal data management procedures.

All field and laboratory data and results will be reviewed, organized, and stored by Third Rock's Data Manager and Sampling Coordinator. In order to accomplish this task, the sampling technician shall submit completed field datasheets and copies of measurements in field notebooks to the Data Manager upon return to the office. The Data Manager will calculate all flows and review the datasheets for completeness. If the sampling technician submits samples to the laboratory, he/she shall obtain a copy of the relinquished COC and submit it to the Data Manager. If the sampling technician relinquishes the COC to the Data Manager, the Data Manager shall similarly obtain a copy of the relinquished COC to retain for recording purposes.

The field data and the COC are stored by the Data Manager until results are received from the analytical laboratory. Hardcopy of the results from the laboratory are reviewed for completeness and for outlier results (i.e. ortho-phosphorus less than total phosphorus, dissolved organic carbon less than total organic carbon, etc). Laboratory results and field measurements are then entered into an electronic "Analytical Monthly Summary" spreadsheet to be submitted, by the Project Administrator, to KDOW once all data for a month is received and entered. Once the "Analytical Monthly Summary" has been submitted to the KDOW, the Data Manager organizes and stores the hardcopies of all information in the designated project folder in the central files.

Third Rock will also deliver analytical data in a COMPASS format to the KDOW as each COC is completed for all sampled stations. The laboratory is responsible to submit the data in the required COMPASS template to the Data Manager once the analytical COC is completed. The Data Manager then enters the field measurements into this database and forwards the database to the Project Administrator. The Project Administrator reviews the file for completeness and then submits the file to the KDOW.

To ensure that data entry is accurate and consistent between the pdf laboratory reports, electronic COMPASS template and the monthly analytical results review, the Data Manager is responsible to hand enter all results from the pdf report into the monthly analytical results review. Using a custom designed verification program within the Access data entry template, a report is generated showing deviations between the COMPASS template and the monthly analytical results. Each deviation is documented and investigated by the Data Manager.

All project related correspondence is documented by an email system. All project related emails are "CC"ed to the Third Rock assigned project file folder for traceability and storage. All other electronic files are stored on a central project drive accessible to the appropriate Third Rock personnel.

3 Assessment and Oversight

3.1 Assessment and Response Actions

Assessment and response actions are necessary to ensure that this QAPP is being implemented as approved. For a general summary of these assessments see Table 4 Dix River Watershed Assessment and Management Reports. The Kentucky Division of Water (KDOW) quality assurance officer (QAO) may freely review all field and laboratory techniques as requested. Any identified problems will be corrected based on recommendations by the QAO. The KDOW will also review analytical results on a monthly basis.

3.1.1 Laboratory Assessments

To ensure conformance with this QAPP and the applicable regulations, certifications, and methods by which the laboratory operates, the laboratory performs several assessment measures. To ensure that the analyst is capable of performing the requested analytical methods to specifications, each analyst is required to acceptably demonstrate this ability prior to conducting sample analyses. The analyst must conduct four replicate analyses of a known standard and achieve precision and accuracy equal to or better than the acceptance ranges for laboratory duplicates and laboratory control samples, respectively.

The laboratory is also required to participate in at least one blind performance evaluation study each year. Performance Evaluation (PE) studies provide an independent assessment of the accuracy of its analyses and maintain laboratory accreditations. All PE analyses performed by the laboratory are performed by the same analysts and using the same procedures that are used for routine sample analyses for the analyte(s) of interest. The PE results must satisfy the PE acceptance criteria specified by the PE provider. After an evaluation of the PE results is received, any results outside of acceptance limits are investigated and corrective actions taken to prevent recurrence of the problem. All findings must be documented and available for review.

The laboratory is also required to have routinely scheduled internal and external audits. The laboratory QA Director or their appointee on an annual basis performs internal audits. Certification bodies usually on a biannual basis perform external audits. In each case, the findings of the audit, both positive and negative are documented, and the corrective response to the cited deviations is required within thirty days of receipt of the audit report. Corrective actions are submitted to the auditing body for review and approval.

3.1.2 Field Assessments

The QA manager is responsible for the overall conformance of Third Rock to the general procedures, protocols, and methods established by this QAPP and internal project related procedures. To ensure overall conformance to this QAPP, the QA manager schedules and manages a weekly status meeting for this project. At this meeting, the status of progress on project related objectives is discussed and

concerns addressed. The Project Administrator is responsible for compiling the minutes of these meetings for review by the QA Manager. These minutes are stored electronically in the project files. The QA Manager may apply spot assessments including supervision of field activities or requests for documentation of the reviews specified herein. The QA Manager may also periodically review the project correspondence files to ensure that all deviations are properly documented and resolved.

To ensure accurate data entry for flow calculations and field data entry into COMPASS templates, all entries and calculations are verified by an independent review. Deviations are documented and corrected accordingly. For those COMPASS entries that are also in the monthly analytical results table, quality assurance is maintained by use of the verification report as in the laboratory data entry.

The Field Logistics Coordinator conducts field procedural audits at the project level. On a quarterly basis, at minimum, the Field Logistics Coordinator will supervise and assess the sampling technicians the following for conformance:

- Calibration and maintenance of field equipment
- Sample collection techniques
- Field measurements and documentation
- Sample handling and custody documentation

The Field Logistics Coordinator will document the review of these items in emails to the Project Administrator. Deviations for the methods specified will be noted, and if necessary, corrective actions will be implemented as specified by the Project Manager. Spot assessments may be applied to ensure that an action is properly corrected. All corrective actions will similarly documented by email correspondence in the project file.

3.2 Reports to Management

Third Rock will prepare a final report that includes the TMDL modeling results and will describe all methods and findings of this project. The final report will satisfy all requirements for the grant.

Prior to the completion of that report, reports on the progress and assessment of the project objectives are produced as summarized in Table 4. All reports are expected to list the personnel or organization responsible for producing the report and the date prepared for traceability purposes.

4 Data Validation and Usability

4.1 Data Review, Verification, and Validation

Initial review of all analytical data is performed by the laboratory against the data quality indicators specified in this QAPP. Corrective actions are taken, if possible while the samples are still within the method specified holding time. Data quality flags are applied to the laboratory results that do not meet these requirements.

Third Rock's Data Manager performs an additional review of the laboratory data as well as the field data. This review, performed within one week of receipt of the results, assesses the completeness and accuracy of the data. Evaluation of the data is made against the DQIs as listed in Table 2. Any data points that seem suspect or require additional analysis are identified during this review. Decisions to reject or additionally qualify the data will be made at the discretion of Third Rock.

4.2 Verification and Validation Methods

The Water Quality Modelers will conduct Third Rock's final review of all data associated with the modeling of the Clarks Run. In this review, they will incorporate all necessary data into a final TMDL document to submit to the KDOW. The final review of all data not associated with this modeling effort will be conducted by the KDOW.

Statistical measures will be used to quantify differences between observed data and model predictions. Such techniques as comparisons of means, regression analysis, and relative error can provide information of model adequacy and error. In addition, model sensitivity analysis will be conducted to determine the effect of model input parameters

The QA Manager will also inspect the final documents to ensure each document is complete and that consistent and appropriate formatting is applied.

4.3 Reconciliation with User Requirements

In the final TMDL document, descriptions of all relevant background information, summary, water body details, monitoring history, current monitoring effort, modeling report, and public involvement will be detailed. Included in this document will be an overall assessment of the data quality and the uncertainty involved in the results.

Load calculations developed from the data will show loads for point sources and nonpoint sources. Example calculations will exhibit the manner in which these loads were calculated. Documentation will be provided for any assumptions made during these calculations, including any data that was rejected or qualified.

In the calculation of the TMDLs specific methodology utilized and any limitations of the model or calculations and of existing data, including data gaps, will be provided.

Based on the model provided by Third Rock, the Division of water will work with the stakeholders in the community to assign the specific load allocations. Margins of Safety are built into assignment of these loads. An implementation plan to reduce the loads will be formulated by KDOW.

5 References

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Assoc., American Water Works Assoc., and Water Pollution Control Federation. 20th Edition. Washington, DC.

Daugherty, R.L., J.B. Franzini, and E.J. Finnemore. 1985. Fluid Mechanics with Engineering Applications. McGraw-Hill, Inc. New York. New York.

Douglas, B. 1958. The ecology of the attached diatoms and other algae in a small stony stream. J. Ecology 46:295-322.

Hydrolab Corporation. 1997. Surveyor 4 Water Quality Data Display User's Manual. Austin, TX.

Karr, J. R. 1981. Assessment of Biotic Integrity using Fish Communities. Fisheries 6:21-27.

Kentucky Division of Water. 2005. 2004 303(d) List of Waters for Kentucky. Natural Resources and Environmental Protection Cabinet, Water Quality Branch. Frankfort, Kentucky.

Kentucky Division of Water. 2002b. Methods for Assessing Biological Integrity of Surface Waters in Kentucky. Natural Resources and Environmental Protection Cabinet, Water Quality Branch. Frankfort, Kentucky.

Kentucky Division of Water. 1997. Reference Reach Fish Community Report. Tech. Report 52. Natural Resources and Environmental Protection Cabinet, Water Quality Branch. Frankfort, Kentucky.

Kentucky Division of Water. 1995. Standard Operating Procedures for Nonpoint Source Surface Water Quality Monitoring Projects. Natural Resources and Environmental Protection Cabinet, Water Quality Branch. Frankfort, Kentucky.

Subcommittee on Sedimentation, Inter-Agency Committee on Water Resources. 1961. A Study of Methods Used in Measurement and Analysis of Sediment Loads in Streams: Report No. 13 The Single-Stage sampler for Suspended Sediment.

U. S. Environmental Protection Agency (USEPA). 1979. Methods for Chemical Analysis of Water and Wastes. Environ. Monit. and Support Lab. Las Vegas, Nevada. EPA/600/4-85/048.

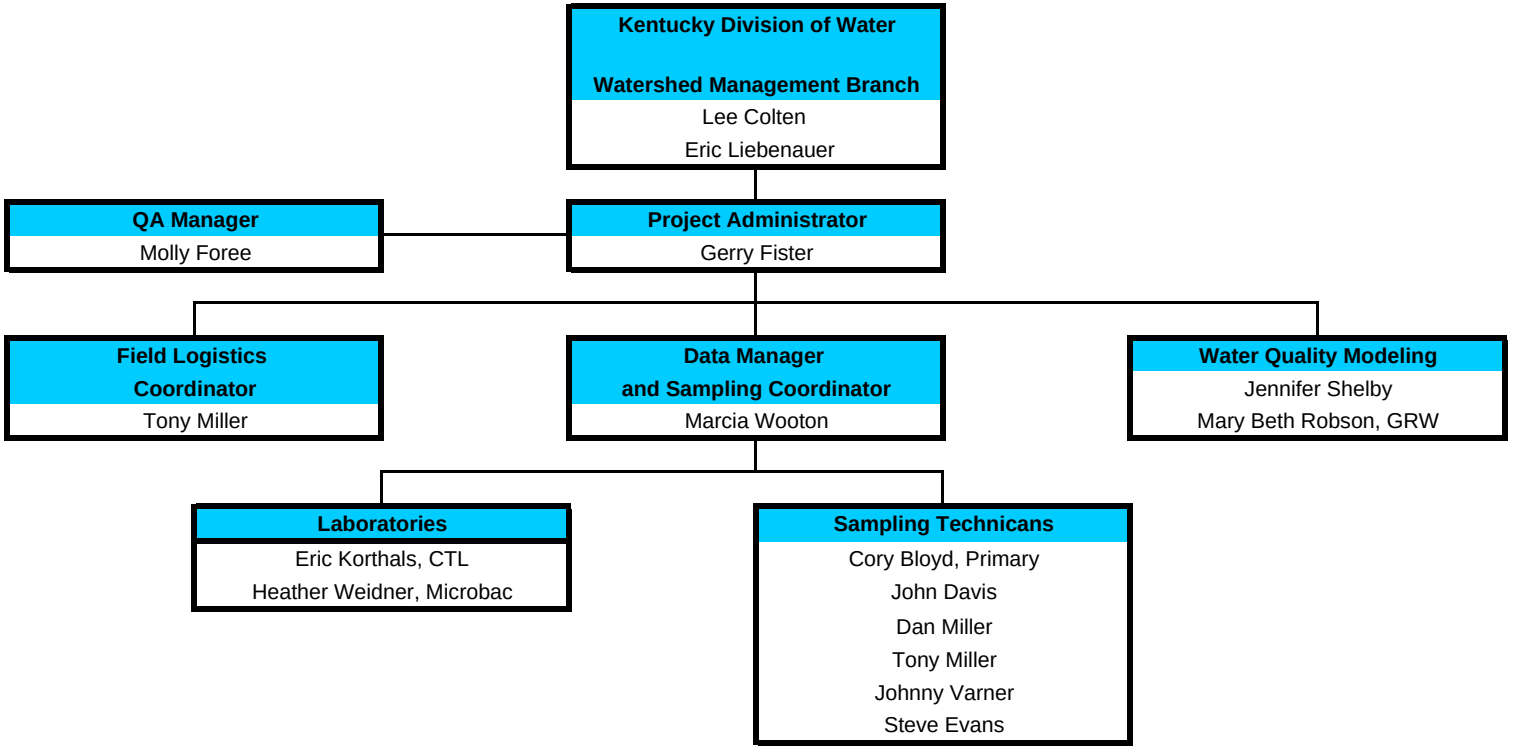
U. S. Geological Survey (USGS). 2000. Modeling Hydrodynamics and Water Quality in Herrington Lake, Kentucky. Water-Resources Investigations Report 99-4281

APPENDICES

APPENDIX A

FIGURE 1:
DIX RIVER ORGANIZATIONAL CHART

Figure 1: Dix River Organizational Chart



APPENDIX B

FIGURE 2:
DIX RIVER PROJECT SCHEDULE

Figure 2: Dix River Project Schedule

Event	Project Schedule
Site Identification and Preparation	January - February 2006
Monitoring and Laboratory Analysis	March 2006 - March 2007
Grab Sampling	March 2006 - March 2007
Passive High Flow Sampling	November 2006 - April 2007
Canopy Coverage	Summer 2006
24 hour Dinural Dissolve Oxygen	Summer 2006
EPA Rapid Bioassessment Protocol	March 2006, March 2007
TMDL modeling on Clarks Run.	April 2007
TMDL model training to KDOW staff	May 2007
Nonpoint Source Pollution Abatement	May 2007

APPENDIX C

FIGURE 3:
WATERSHED OVERVIEW MAP

Map Document: (P:\2005\5167-KDOW-DixRiver\Mapping\GIS\watershed_overview.mxd) 3/29/2006 -- 10:02:55 AM WCO

Dix River Watershed

Clark's Run Watershed

Hanging Fork Watershed

MERCER

BOYLE

JESSAMINE

GARRARD

MADISON

LINCOLN

CASEY

ROCKCASTLE

Wilmore

Nicholasville

Lexington-Fayette

Harrodsburg

Burgin

Danville

Junction City

Richmond

Berea

Lancaster

Stanford

Crab Orchard

Brodhead

Mount Vernon

Herrington Lake

Clark's Run

Hanging Fork

Dix River

US-127

US-68

US-27

US-25

US-150

KY-52

I-75

KY 70

Rocky Fork

Cane Run

Tanyard Branch

McKecknie Creek

Mocks Branch

Spears Creek

Clarks Run

Bals Branch

Knoblick Creek

White Oak Creek

Harris Creek

Bannister Creek

Frog Branch

Hanging Fork Creek

Blue Lick Creek

Peyton Creek

Martins Branch

Hawkins Branch

Logan Creek

Neals Creek

Dix River

Drakes Creek

East Fork Drakes Creek

Harmons Lick

Indian Branch

Mudlick Branch

Fall Lick

North Fork Copper Creek

Copper Creek

Crane Branch

Cedar Creek

Turkey Creek

Gilberts Creek

Stingy Creek

Grassy Branch

Long Branch

Bowman Branch

Tarkin Branch

Negro Creek

Little Negro Creek

Boone Fork

SHEET
1 OF 1

0 1.5 3 6 Miles

Watershed Overview

Kentucky UNBRIDLED SPIRIT

THIRD ROCK CONSULTANTS

APPENDIX D

FIGURE 4:
HANGING FORK AND CLARKS RUN MAP

Hanging Fork and Clarks Run

SHEET 1 OF 1

0 0.5 1 2 Miles

Kentucky UNBRIDLED SPIRIT

THIRD ROCK CONSULTANTS

Sample Stations
Discharge Locations
Streams
Hanging Fork Watershed
Clarks Run Watershed
Dix River Watershed
Herrington Lake

305b Status of Streams/Lakes
Fully
Not supporting
Partial
Threatened

Chenault Bridge
Herrington Lake
DOW Dix
DOW Clark's Run
Clarks Run KY 52
Balls Branch Mouth
Hanging Fork Mouth
South Second Street Clarks Run
Clark's Run By-Pass
Clark's Run KY 150
Corporate Drive
Ball's Branch West
Hanging Fork KY 150
Junction City
Oak Creek
Knoblick Creek
Moores Lane
McCormick Church
Blue Lick Creek
Frog Branch
Peyton Creek
Chicken Bristle
McKinney Branch
West Hustonville
Baughman Creek
Hanging Fork
Stanford
Stanford Reservoir

Dix River
Clarks Run
Boone Creek
Spears Creek
Mocks Branch
Balls Branch
White Oak Creek
Knoblick Creek
Hawkins Branch
Logan Creek
Neals Creek
Harris Creek
Hanging Fork Creek
Baughman Creek
Peyton Creek
Martins Branch
McKinney Branch

US 150
US 127
US 127
US 150
US 127

Map Document: (P:\2005\5167.KDOW\DryRiver\Mapping\GIS\meeting_map.mxd) 3/29/2006 - 7:37:13 AM WCO

APPENDIX E

FIGURE 5:
DIX RIVER MAP

Dix River

Herrington Lake
DOW Dix
Hanging Fork Mouth
Above Hanging Fork Dix
White Oak Creek
Gilbert's Creek
Logan Creek
Stanford Reservoir
Drakes Creek
Cedar Creek
Dix Crab Orchard
Copper Creek
Gum Sulphur
Brodhead

Dix River
Boone Creek
White Oak Creek
Gilberts Creek
Turkey Creek
Hawkins Branch
Logan Creek
Neals Creek
Walnut Flat Creek
Cedar Creek
Sinking Creek
Drakes Creek
East Fork Drakes Creek
Flax Creek
Harmons Lick
Indian Branch
Fall Lick
Crane Branch
Copper Creek
North Fork Copper Creek
Grassy Branch
Long Branch
Bowman Branch
Tarkin Branch
Negro Creek
Boone Fork
Little Negro Creek

US 27
US 150
KY 52
KY 70

Lancaster
Stanford
Crab Orchard
Brodhead

23, 24
21
50, 51
25, 26, 27
0
40, 41
33, 34

N
W E
S

Sample Stations
Discharge Locations
Streams
Hanging Fork Watershed
Clarks Run Watershed
Dix River Watershed
Herrington Lake

305b Status of Streams/Lakes

Fully
Not supporting
Partial
Threatened

SHEET 1 OF 1

0 0.5 1 2 Miles

Kentucky UNBRIDLED SPIRIT

THIRD ROCK CONSULTANTS

Map Document: (P:\2005\5167-KDOW-DixRiver\Mapping\GIS\meeting_map_dix.mxd) 3/29/2006 -- 7:50:23 AM WCO

APPENDIX F

FIGURE 6:
EPA RAPID BIOASSESSMENT PROTOCOL (RBP) WORKSHEET

DIX RIVER PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET

Station ID:	**Stream Name:**	**Project #:**
Station type (select/nonselect):	**Watershed:**	**Form Completed by:**
Collection Date/Time:	**Investigators:**	**Location:**

Picture #s:

WEATHER CONDITIONS

Now

- ☐ storm (heavy rain)
- ☐ rain (steady rain)
- ☐ showers (intermittent)
- ___% ☐ % cloud cover
- ☐ clear/sunny

Past 24 Hours

- ☐
- ☐
- ☐
- ☐ ____%
- ☐

Has there been a heavy rain in the last 7 days? ☐ Yes ☐ No

Air Temperature _____°F

Other_____

STREAM CHARACTERIZATION

Stream Subsystem ☐ Perennial ☐ Intermittent

Estimate # of intermittent tributaries above this station _____

Do the tributaries appear to contribute to any NPS pollution? _____

If yes, explain: _____

INSTREAM FEATURES

Estimated Reach Length _____ yards

Estimated Stream Width:
Pools:_____ Runs:_____ Riffles:_____

High Water Mark: _____ ft

Estimated Stream Depth:
Pools:_____ Runs:_____ Riffles:_____

Channelized ☐ Yes ☐ No

Stream Flow:
☐ Flooding ☐ Bankful ☐ High ☐ Normal
☐ Low ☐ Pooled ☐ Dry

Proportion of reach represented by Morphology Types
☐ Riffle_____% ☐ Run _____%
☐ Pool _____%

AQUATIC VEGETATION/FUNGUS

Indicate the dominant type and record the dominant species present
☐ Rooted emergent ☐ Rooted submergent ☐ Rotted floating ☐ Free floating
☐ Floating Algae ☐ Attached Algae

Indicate the macrohabitats sampled for periphyton:
☐ Riffle ☐ Run ☐ Pool

Indicate the microhabitat sampled for periphyton and its relative proportion:
Rocks_____ Woody Debris ____ Bedrock ____ Vegetation ____ Artificial Substrate ____ Other ____

Estimate periphyton coverage:
☐ Dense (>75%) ☐ Moderate (50-75%) ☐ Sparse (15-50%) ☐ Absent (<15%)

Is the periphyton coverage consistent over entire reach? ____

If no, describe differences in bottom coverage:

Is sewage fungus preset?
☐ Yes ☐ No

Describe the extent of the fungus coverage:
☐ Dense (>75%) ☐ Moderate (50-75%) ☐ Sparse (15-50%) ☐ Absent (<15%)

Describe the extent of organic sediment accumulation:
☐ Dense (>75%) ☐ Moderate (50-75%) ☐ Sparse (15-50%) ☐ Absent (<15%)

.

WATER QUALITY	**Temperature**_____°F **Specific Conductance**_____µS/cm **Dissolved Oxygen** _____mg/L, _____% Sat **pH**_____ (Standard Units) **Turbidity** _____NTU **WQ Instrument Used**_____ ☐ Hydrolab MS5 ☐ Hydrolab Quanta ☐ Lamotte 2020 (turb) ☐ Other_____ **Water Odors** ☐ Normal/None ☐ Sewage ☐ Petroleum ☐ Chemical ☐ Fishy ☐ Other _____ **Water Surface Oils** ☐ Slick ☐ Sheen ☐ Globs ☐ Flecks ☐ None ☐ Other _____ **Turbidity (if not measured)** ☐ Clear ☐ Slightly Turbid ☐ Turbid ☐ Opaque ☐ Stained ☐ Other _____
SEDIMENT/ SUBSTRATE	**Odors** ☐ Normal ☐ Sewage ☐ Petroleum ☐ Chemical ☐ Anaerobic ☐ None ☐ Other _____ **Oils** ☐ Absent ☐ Slight ☐ Moderate ☐ Profuse **Sedimentation:** ☐ Heavy ☐ Moderate ☐ Slight ☐ None **Deposits** ☐ Sludge ☐ Sawdust ☐ Paper Fiber ☐ Sand ☐ Relict Shells ☐ Other _____ **Looking at stones which are not deeply embedded, are the undersides black in color?** ☐ Yes ☐ No

Modified RBP Worksheet

Riparian Vegetation: Dominate Type: Dom. Tree/Shrub Taxa: ❑ Trees ❑ Shrubs ❑ Grasses ❑ Herbaceous Number of strata ____	Canopy Cover: ❑ Fully Exposed (0-25%) ❑ Partially Exposed (25-50%) ❑ Partially Shaded (50-75%) ❑ Fully Shaded (75-100%)	**Note the approximate length of stream that is affected by the following:** Stream diversion_____ Stream straightening_____ Concrete streambank/bottom_____

Substrate ❑Est. ❑P.C.	**Riffle_____%**	**Run_____%**	**Pool_____%**
Silt/Clay (<0.06 mm)			
Sand (0.06 – 2 mm)			
Gravel (2-64 mm)			
Cobble (64 – 256 mm)			
Boulders (>256 mm)			
Bedrock			

Habitat	**Condition Category**			
Parameter	**Optimal**	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Sow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr.) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8.Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ____ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ____ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score

LAND USES IN THE WATERSHED

1. Specific uses identified (check as many as apply)

	Streamside	100—200 Yards
Residential:		
Single-family housing	.	. .
Apartment building	.	. .
Lawns	.	. .
Playground	.	. .
Parking lot	.	. .
Other _____	.	. .
Commercial / Industrial / Institutional:		
Commercial development	.	. .
(stores, restaurants)	.	. .
Auto repair/gas station	.	. .
Factory/Power plant	.	. .
Sewage treatment facility	.	. .
Water treatment facility	.	. .
Institution (e.g., school, offices)	.	. .
Landfill	.	. .
Automobile graveyard	.	. .
Bus or taxi depot	.	. .
Other _____	.	. .
Forest / Parkland:		
Recreational park	.	. .
National/State Forest	.	. .
Woods/Greenway	.	. .
Other _____	.	. .
Agricultural / Rural:		
Grazing land	.	. .
Cropland	.	. .
Animal feedlot	.	. .
Isolated farm	.	. .
Old (abandoned) field	.	. .
Fish hatchery	.	. .
Tree farm	.	. .
Other _____	.	. .

2. Additional activities in the watershed (check as many as apply)

	Streamside	100—200 Yards
Construction	.	. .
Building construction	.	. .
Roadway	.	. .
Bridge construction	.	. .
Other _____	.	. .
Logging		
Selective logging	.	. .
Intensive logging	.	. .
Lumber treatment facility	.	. .
Other _____	.	. .
Mining		
Strip mining	.	. .
Pit mining	.	. .
Abandoned mine	.	. .
Quarry	.	. .
Other _____	.	. .

Recreation

Biking/Off-road vehicle trails . . .
Horseback riding trail . . .
Boat ramp . . .
Jogging paths/hiking trail . . .
Swimming area . . .
Fishing area . . .
Picnic area . . .
Golf course . . .
Campground/trailer park . . .
Power boating . . .
Other _____ . . .

VELOCITY MEASUREMENT DATA

Infinity Depth and Time:

Notes: **LEOW =** **REOW =** **DEPTH =**

** 0 = Left Bank (when looking downstream)

Distance from L Bank (ft)	Total Depth (ft)	Depth of Avg. Velocity (0.6, 0.2, or 0.8D)	Starting Count	Ending Count	Time (~1min)	Notes
						Total Stream Discharge (ft^3/sec) =

* Stand at least 1' downstream of meter

* When D<2.5', avg V occurs at 0.6D

* When D>2.5', measure V at 0.2D and 0.8D (then will average these values) Updated 5/10/06 mlw

APPENDIX G

FIGURE 7:
DATA CHARACTERIZATION AND WATER QUALITY DATASHEETS

DIX RIVER PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET

Station ID:	**Stream Name:**	**Project #:**
Station type (select/nonselect):	**Watershed:**	**Form Completed by:**
Collection Date/Time:	**Investigators:**	**Location:**

Picture #s:

WEATHER CONDITIONS

Now / **Past 24 Hours**

- ☐ storm (heavy rain) ☐
- ☐ rain (steady rain) ☐
- ☐ showers (intermittent) ☐
- ____% ☐ % cloud cover ☐ ____%
- ☐ clear/sunny ☐

Has there been a heavy rain in the last 7 days? ☐ Yes ☐ No

Air Temperature _____°F

Other_____

STREAM CHARACTERIZATION

Stream Subsystem
☐ Perennial ☐ Intermittent

Estimate # of intermittent tributaries above this station _____

Do the tributaries appear to contribute to any NPS pollution? _____

If yes, explain: _____

INSTREAM FEATURES

Estimated Reach Length _____ yards

Estimated Stream Width:
Pools:_____ Runs:_____ Riffles:_____

High Water Mark: _____ ft

Estimated Stream Depth:
Pools:_____ Runs:_____ Riffles:_____

Channelized ☐ Yes ☐ No

Proportion of reach represented by Morphology Types
☐ Riffle_____% ☐ Run _____%
☐ Pool _____%

Stream Flow:
☐ Flooding ☐ Bankful ☐ High ☐ Normal
☐ Low ☐ Pooled ☐ Dry

AQUATIC VEGETATION/FUNGUS

Indicate the dominant type and record the dominant species present
☐ Rooted emergent ☐ Rooted submergent ☐ Rotted floating ☐ Free floating
☐ Floating Algae ☐ Attached Algae

Indicate the macrohabitats sampled for periphyton:
☐ Riffle ☐ Run ☐ Pool

Indicate the microhabitat sampled for periphyton and its relative proportion:
Rocks_____ Woody Debris ____ Bedrock ____ Vegetation ____ Artificial Substrate ____ Other ____

Estimate periphyton coverage:
☐ Dense (>75%) ☐ Moderate (50-75%) ☐ Sparse (15-50%) ☐ Absent (<15%)

Is the periphyton coverage consistent over entire reach? ____

If no, describe differences in bottom coverage:

Is sewage fungus preset?
☐ Yes ☐ No

Describe the extent of the fungus coverage:
☐ Dense (>75%) ☐ Moderate (50-75%) ☐ Sparse (15-50%) ☐ Absent (<15%)

Describe the extent of organic sediment accumulation:
☐ Dense (>75%) ☐ Moderate (50-75%) ☐ Sparse (15-50%) ☐ Absent (<15%)

.

WATER QUALITY	**Temperature**_____°F **Specific Conductance**_____µS/cm **Dissolved Oxygen** _____mg/L, _____% Sat **pH**_____ (Standard Units) **Turbidity** _____NTU **WQ Instrument Used**_____ ☐ Hydrolab MS5 ☐ Hydrolab Quanta ☐ Lamotte 2020 (turb) ☐ Other_____ **Water Odors** ☐ Normal/None ☐ Sewage ☐ Petroleum ☐ Chemical ☐ Fishy ☐ Other _____ **Water Surface Oils** ☐ Slick ☐ Sheen ☐ Globs ☐ Flecks ☐ None ☐ Other _____ **Turbidity (if not measured)** ☐ Clear ☐ Slightly Turbid ☐ Turbid ☐ Opaque ☐ Stained ☐ Other _____
SEDIMENT/ SUBSTRATE	**Odors** ☐ Normal ☐ Sewage ☐ Petroleum ☐ Chemical ☐ Anaerobic ☐ None ☐ Other _____ **Oils** ☐ Absent ☐ Slight ☐ Moderate ☐ Profuse **Sedimentation:** ☐ Heavy ☐ Moderate ☐ Slight ☐ None **Deposits** ☐ Sludge ☐ Sawdust ☐ Paper Fiber ☐ Sand ☐ Relict Shells ☐ Other _____ **Looking at stones which are not deeply embedded, are the undersides black in color?** ☐ Yes ☐ No

VELOCITY MEASUREMENT DATA

Infinity Depth and Time:

Notes: **LEOW =** **REOW =** **DEPTH =**

** 0 = Left Bank (when looking downstream)

Distance from L Bank (ft)	Total Depth (ft)	Depth of Avg. Velocity (0.6, 0.2, or 0.8D)	Starting Count	Ending Count	Time (~1min)	Notes
						Total Stream Discharge (ft^3/sec) =

* Stand at least 1' downstream of meter

* When D<2.5', avg V occurs at 0.6D

* When D>2.5', measure V at 0.2D and 0.8D (then will average these values) Updated 5/10/06 mlw

APPENDIX H

FIGURE 8:
CHAIN-OF-CUSTODY FORMS

COC# | **CHAIN OF CUSTODY** | **PAGE 1 OF 1**

Client:

Project Name:

Project #:

Collected By: Third Rock Consultants -

Third Rock Consultants Project Contact: Marcia L. Wooton

Third Rock Consultants Phone #: 859-977-2000

Third Rock Consultants

COMPASS Reporting

Project Code/Short Name: HERTMDL

Medium: Water - ambient surface

Sample Purpose Description: Sampling effort to collect nutrients, pathogens, and other water quality data in Herrington Lake and associated tributaries.

EXAMPLE Chain of Custody

(customized per event i.e. watershed, parameters, laboratory specifics, etc.)

								* * Preservation Type									* * Preservation Code
								-	-	-	SA	SA	-	SH/ZA			AA - Ascrobic Acid AC - NH4Cl E - EnCore HA - HCl M - Methanol NA - HNO3 SA - H2SO4 SH - NaOH SS - Na2SO3 ST - Na2S2O3 ZA - Zinc Acetate O - Other _____
								Requested Analysis									
								CBOD5, TSS, SO4	CBOD5, SO4	TSS, SO4	TKN, NH3, P-T	TOC	P-O, NO2, NO3	Sulfide			
Sample I.D.	Station Name	County	Zone-Depth	Collection Date	Collection Time	Grab / Comp	Filt'd Y/N	32oz P	32oz P	32oz P	8oz P	4oz P	16oz P	16oz P			Lab #	Comments

Reliquished By:	Date/Time	Received By:	Date/Time

Properly Preserved: Yes / No

Bottles Intact: Yes / No

Temp. @ Receipt: _____ °C By: _____

Laboratory: ADD "day", highlighted in yellow, to sample id (without any spaces).

COMPASS Reporting Notes: Previous information provided for Project Level Data Description is now the Sample Purpose Description; Project Level Data Description field is now for Case Narrative from laboratory.

APPENDIX I

FIGURE 9:
ANALYTICAL LABORATORY REPORTS

EnviroData Group
Accredited Lab Data for Today's Environment

Microbac

2520 Regency Rd.
Lexington, KY 40503
Phone: 859-276-3506
Toll Free: 800-489-3506
Fax: 859-278-5665
E-mail: info@envirodatagroup.com

Analytical Results

Third Rock Consultants
Attn: Marcia Wooton
2514 Regency Rd

Lexington, KY 40503

cc: pdf

Chain of Custody: 45643
Project Name: Dix River TMDL-Hanging Fork
Project Number: 5167
Report Reference:45643-20060426103701

Date/Time Received: 04/13/2006 09:05
Temperature Upon Receipt: 2 C

Collector: Client
Client Manager: Heather Weidner

Laboratory Sample #: 482663 **Client Sample ID:** Chicken Bristle **Sampled:** 04/12/2006 13:45
Sample Replicate # 1

Biochemical Oxygen Demand-Carbonaceous **Method:** EPA 405.1 **Prep. Method:** N/A
Analyzed by CDP on April 14, 2006 at 08:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Oxygen Demand, Biochemical, 5-Day/	< 2.00	mg/L	2.00	N/A	

Total Coliform **Method:** SM9223 **Prep. Method:** N/A
Analyzed by TWL on April 13, 2006 at 15:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Total Coliform	> 2,010	MPN	0	N/A	D
Ecoli	360	MPN	0	N/A	D

Specific Conductance (Field) **Method:** EPA120.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 13:45.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Specific Conductance (Field)	302.0	umhos/cm	N/A	N/A	

Dissolved Oxygen (Field) **Method:** EPA360.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 13:45.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Dissolved Oxygen (Field)	13.88	mg/L	N/A	N/A	

pH (Field) **Method:** EPA150.1/SW9045 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 13:45.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
pH (Field)	8.55	S.U.	N/A	N/A	

Temperature F (field) **Method:** EPA170.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 13:45.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Temperature (Field)	61.9	Fahrenheit	N/A	N/A	

Turbity (Field) **Method:** **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 13:45.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Turbidity	NA			N/A	

Results reported on a Wet Weight Basis

Issued: 4/26/2006

Laboratory Sample #: 482663 **Client Sample ID:** Chicken Bristle **Sampled:** 04/12/2006 13:45
Sample Replicate # 1

Inorganic Anions **Method:** EPA 300 **Prep. Method:** N/A
Analyzed by KTL on April 14, 2006 at 11:05.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Nitrite	< 0.150	MG/L	0.15	N/A	
Nitrogen, Nitrate	1.30	MG/L	0.11	N/A	

Carbon, Total Organic Sub **Method:** N/A **Prep. Method:** N/A
Analyzed by SUB LAB on at .

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Carbon, Total Organic	2.00	mg/L	N/A	N/A	

Ammonia Nitrogen **Method:** EPA 350.1 **Prep. Method:** N/A
Analyzed by JEE on April 18, 2006 at 10:33.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Ammonia	< 0.100	mg/L	0.100	N/A	

Ortho-Phosphate Phosphorus **Method:** EPA 365.2 **Prep. Method:** N/A
Analyzed by JPM on April 14, 2006 at 09:55.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Ortho-Phosphate	0.033	mg/L as P	0.010	N/A	

Total Phosphorus **Method:** EPA 365.1 **Prep. Method:** EPA365.1
Analyzed by JPM on April 14, 2006 at 14:51. Prepped by JPM on April 14, 2006 at 10:50.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Total	0.039	mg/L as P	0.010	N/A	

Total Kjeldahl Nitrogen **Method:** EPA 351.2 **Prep. Method:** EPA 351.2
Analyzed by JPM on April 18, 2006 at 16:16. Prepped by JPM on April 18, 2006 at 11:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Total Kjeldahl	0.259	mg/L	0.100	N/A	

Total Suspended Solids **Method:** EPA 160.2/160.4 **Prep. Method:** N/A
Analyzed by KTL on April 17, 2006 at 18:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Solids, Total Suspended	< 5.00	MG/L	5	N/A	

Total Alkalinity **Method:** EPA 310.1 **Prep. Method:** N/A
Analyzed by JEE on April 14, 2006 at 12:15.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Alkalinity, Total	131	mg/L CaCO3	5.00	N/A	

Laboratory Sample #: 482667 **Client Sample ID:** Peyton Creek **Sampled:** 04/12/2006 15:00
Sample Replicate # 1

Total Coliform **Method:** SM9223 **Prep. Method:** N/A
Analyzed by TWL on April 13, 2006 at 15:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Total Coliform	> 2,010	MPN	0	N/A	D
Ecoli	1,650	MPN	0	N/A	D

Laboratory Sample #: 482667 **Client Sample ID:** Peyton Creek **Sampled:** 04/12/2006 15:00
Sample Replicate # 1

Specific Conductance (Field) **Method:** EPA120.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 15:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Specific Conductance (Field)	327.1	umhos/cm	N/A	N/A	

Dissolved Oxygen (Field) **Method:** EPA360.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 15:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Dissolved Oxygen (Field)	11.91	mg/L	N/A	N/A	

pH (Field) **Method:** EPA150.1/SW9045 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 15:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
pH (Field)	8.63	S.U.	N/A	N/A	

Temperature F (field) **Method:** EPA170.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 15:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Temperature (Field)	67.5	Fahrenheit	N/A	N/A	

Turbity (Field) **Method:** **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 15:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Turbidity	NA			N/A	

Inorganic Anions **Method:** EPA 300 **Prep. Method:** N/A
Analyzed by KTL on April 14, 2006 at 12:53.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Nitrite	< 0.150	MG/L	0.15	N/A	
Nitrogen, Nitrate	2.40	MG/L	0.11	N/A	

Carbon, Total Organic Sub **Method:** N/A **Prep. Method:** N/A
Analyzed by SUB LAB on at .

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Carbon, Total Organic	1.90	mg/L	N/A	N/A	

Ammonia Nitrogen **Method:** EPA 350.1 **Prep. Method:** N/A
Analyzed by JEE on April 18, 2006 at 10:35.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Ammonia	< 0.100	mg/L	0.100	N/A	

Ortho-Phosphate Phosphorus **Method:** EPA 365.2 **Prep. Method:** N/A
Analyzed by JPM on April 14, 2006 at 09:57.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Ortho-Phosphate	0.069	mg/L as P	0.010	N/A	

Total Phosphorus **Method:** EPA 365.1 **Prep. Method:** EPA365.1
Analyzed by JPM on April 14, 2006 at 14:52. Prepped by JPM on April 14, 2006 at 10:50.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Total	0.080	mg/L as P	0.010	N/A	

Laboratory Sample #: 482667 **Client Sample ID:** Peyton Creek **Sampled:** 04/12/2006 15:00
Sample Replicate # 1

Total Kjeldahl Nitrogen **Method:** EPA 351.2 **Prep. Method:** EPA 351.2
Analyzed by JPM on April 18, 2006 at 16:17. Prepped by JPM on April 18, 2006 at 11:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Total Kjeldahl	0.552	mg/L	0.100	N/A	

Total Suspended Solids **Method:** EPA 160.2/160.4 **Prep. Method:** N/A
Analyzed by KTL on April 17, 2006 at 18:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Solids, Total Suspended	7.00	MG/L	5	N/A	

Laboratory Sample #: 482668 **Client Sample ID:** McKinney Branch **Sampled:** 04/12/2006 12:30
Sample Replicate # 1

Total Coliform **Method:** SM9223 **Prep. Method:** N/A
Analyzed by TWL on April 13, 2006 at 15:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Total Coliform	> 2,010	MPN	0	N/A	D
Ecoli	590	MPN	0	N/A	D

Specific Conductance (Field) **Method:** EPA120.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 12:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Specific Conductance (Field)	399.2	umhos/cm	N/A	N/A	

Dissolved Oxygen (Field) **Method:** EPA360.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 12:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Dissolved Oxygen (Field)	12.04	mg/L	N/A	N/A	

pH (Field) **Method:** EPA150.1/SW9045 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 12:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
pH (Field)	8.41	S.U.	N/A	N/A	

Temperature F (field) **Method:** EPA170.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 12:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Temperature (Field)	59.7	Fahrenheit	N/A	N/A	

Turbity (Field) **Method:** **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 12:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Turbidity	NA			N/A	

Inorganic Anions **Method:** EPA 300 **Prep. Method:** N/A
Analyzed by KTL on April 14, 2006 at 12:55.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Nitrite	< 0.150	MG/L	0.15	N/A	
Nitrogen, Nitrate	1.90	MG/L	0.11	N/A	

Laboratory Sample #: 482668 **Client Sample ID:** McKinney Branch **Sampled:** 04/12/2006 12:30
Sample Replicate # 1

Carbon, Total Organic Sub **Method:** N/A **Prep. Method:** N/A
Analyzed by SUB LAB on at .

Carbon, Total Organic	2.00	mg/L	N/A	N/A

Ammonia Nitrogen **Method:** EPA 350.1 **Prep. Method:** N/A
Analyzed by JEE on April 18, 2006 at 10:38.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Ammonia	< 0.100	mg/L	0.100	N/A	

Ortho-Phosphate Phosphorus **Method:** EPA 365.2 **Prep. Method:** N/A
Analyzed by JPM on April 14, 2006 at 09:58.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Ortho-Phosphate	0.068	mg/L as P	0.010	N/A	

Total Phosphorus **Method:** EPA 365.1 **Prep. Method:** EPA365.1
Analyzed by JPM on April 14, 2006 at 14:53. Prepped by JPM on April 14, 2006 at 10:50.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Total	0.076	mg/L as P	0.010	N/A	

Total Kjeldahl Nitrogen **Method:** EPA 351.2 **Prep. Method:** EPA 351.2
Analyzed by JPM on April 18, 2006 at 16:18. Prepped by JPM on April 18, 2006 at 11:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Total Kjeldahl	0.371	mg/L	0.100	N/A	

Total Suspended Solids **Method:** EPA 160.2/160.4 **Prep. Method:** N/A
Analyzed by KTL on April 17, 2006 at 18:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Solids, Total Suspended	< 5.00	MG/L	5	N/A	

Laboratory Sample #: 482669 **Client Sample ID:** Baughman Creek **Sampled:** 04/12/2006 10:00
Sample Replicate # 1

Total Coliform **Method:** SM9223 **Prep. Method:** N/A
Analyzed by TWL on April 13, 2006 at 15:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Total Coliform	> 2,010	MPN	0	N/A	D
Ecoli	340	MPN	0	N/A	D

Specific Conductance (Field) **Method:** EPA120.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Specific Conductance (Field)	275.9	umhos/cm	N/A	N/A	

Dissolved Oxygen (Field) **Method:** EPA360.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Dissolved Oxygen (Field)	11.28	mg/L	N/A	N/A	

pH (Field) **Method:** EPA150.1/SW9045 **Prep. Method:** N/A

Laboratory Sample #: 482669 **Client Sample ID:** Baughman Creek **Sampled:** 04/12/2006 10:00
Sample Replicate # 1

pH (Field) **Method:** EPA150.1/SW9045 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
pH (Field)	8.11	S.U.	N/A	N/A	

Temperature F (field) **Method:** EPA170.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Temperature (Field)	54.6	Fahrenheit	N/A	N/A	

Turbity (Field) **Method:** **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Turbidity	NA			N/A	

Inorganic Anions **Method:** EPA 300 **Prep. Method:** N/A
Analyzed by KTL on April 14, 2006 at 12:56.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Nitrite	< 0.150	MG/L	0.15	N/A	
Nitrogen, Nitrate	1.30	MG/L	0.11	N/A	

Carbon, Total Organic Sub **Method:** N/A **Prep. Method:** N/A
Analyzed by SUB LAB on at .

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Carbon, Total Organic	1.90	mg/L	N/A	N/A	

Ammonia Nitrogen **Method:** EPA 350.1 **Prep. Method:** N/A
Analyzed by JEE on April 18, 2006 at 10:43.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Ammonia	< 0.100	mg/L	0.100	N/A	

Ortho-Phosphate Phosphorus **Method:** EPA 365.2 **Prep. Method:** N/A
Analyzed by JPM on April 14, 2006 at 09:59.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Ortho-Phosphate	0.081	mg/L as P	0.010	N/A	

Total Phosphorus **Method:** EPA 365.1 **Prep. Method:** EPA365.1
Analyzed by JPM on April 14, 2006 at 14:54. Prepped by JPM on April 14, 2006 at 10:50.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Total	0.065	mg/L as P	0.010	N/A	

Total Kjeldahl Nitrogen **Method:** EPA 351.2 **Prep. Method:** EPA 351.2
Analyzed by JPM on April 18, 2006 at 16:19. Prepped by JPM on April 18, 2006 at 11:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Total Kjeldahl	0.530	mg/L	0.100	N/A	

Total Suspended Solids **Method:** EPA 160.2/160.4 **Prep. Method:** N/A
Analyzed by KTL on April 17, 2006 at 18:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Solids, Total Suspended	< 5.00	MG/L	5	N/A	

Laboratory Sample #: 482670 **Client Sample ID:** West Hustonville **Sampled:** 04/12/2006 11:15
Sample Replicate # 1

Total Coliform **Method:** SM9223 **Prep. Method:** N/A
Analyzed by TWL on April 13, 2006 at 15:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Total Coliform	> 2,010	MPN	0	N/A	D
Ecoli	530	MPN	0	N/A	D

Specific Conductance (Field) **Method:** EPA120.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 11:15.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Specific Conductance (Field)	237.7	umhos/cm	N/A	N/A	

Dissolved Oxygen (Field) **Method:** EPA360.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 11:15.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Dissolved Oxygen (Field)	13.01	mg/L	N/A	N/A	

pH (Field) **Method:** EPA150.1/SW9045 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 11:15.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
pH (Field)	8.57	S.U.	N/A	N/A	

Temperature F (field) **Method:** EPA170.1 **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 11:15.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Temperature (Field)	55.7	Fahrenheit	N/A	N/A	

Turbity (Field) **Method:** **Prep. Method:** N/A
Analyzed by FIELD on April 12, 2006 at 11:15.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Turbidity	NA			N/A	

Inorganic Anions **Method:** EPA 300 **Prep. Method:** N/A
Analyzed by KTL on April 14, 2006 at 12:57.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Nitrite	< 0.150	MG/L	0.15	N/A	
Nitrogen, Nitrate	1.10	MG/L	0.11	N/A	

Carbon, Total Organic Sub **Method:** N/A **Prep. Method:** N/A
Analyzed by SUB LAB on at .

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Carbon, Total Organic	1.80	mg/L	N/A	N/A	

Ammonia Nitrogen **Method:** EPA 350.1 **Prep. Method:** N/A
Analyzed by JEE on April 18, 2006 at 10:45.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Ammonia	< 0.100	mg/L	0.100	N/A	

Ortho-Phosphate Phosphorus **Method:** EPA 365.2 **Prep. Method:** N/A
Analyzed by JPM on April 14, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers

EnviroData Group

Accredited Lab Data for Today's Environment

Microbac

Chain of Custody: 45643
Project Name: Dix River TMDL-Hanging Fork
Project Number: 5167

Laboratory Sample #: 482670 **Client Sample ID:** West Hustonville **Sampled:** 04/12/2006 11:15
Sample Replicate # 1

Ortho-Phosphate Phosphorus **Method:** EPA 365.2 **Prep. Method:** N/A
Analyzed by JPM on April 14, 2006 at 10:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Ortho-Phosphate	0.017	mg/L as P	0.010	N/A	

Total Phosphorus **Method:** EPA 365.1 **Prep. Method:** EPA365.1
Analyzed by JPM on April 14, 2006 at 14:55. Prepped by JPM on April 14, 2006 at 10:50.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Phosphorus, Total	0.019	mg/L as P	0.010	N/A	

Total Kjeldahl Nitrogen **Method:** EPA 351.2 **Prep. Method:** EPA 351.2
Analyzed by JPM on April 18, 2006 at 16:22. Prepped by JPM on April 18, 2006 at 11:30.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Nitrogen, Total Kjeldahl	0.403	mg/L	0.100	N/A	

Total Suspended Solids **Method:** EPA 160.2/160.4 **Prep. Method:** N/A
Analyzed by KTL on April 17, 2006 at 18:00.

Parameter	Result	Units	Reporting Limit	Client Limit	Qualifiers
Solids, Total Suspended	< 5.00	MG/L	5	N/A	

All samples were received intact and properly preserved unless otherwise noted.
The results reported relate only to the samples tested.
This report shall not be reproduced except in full, without written approval of this laboratory.

ACCREDITED
Lab#: 100343

Submitted by: Heather J Weidner

Client Manager: Heather Weidner
Please contact Heather Weidner with any questions.

Specific tests covered by the A2LA accreditation meet the requirements of the A2LA accreditation standard. Please refer to http://www.envirodatagroup.com/EDG_A2LA_Accredited_Analytes.pdf on our website for a list of our current A2LA accreditations.

EnviroData Group

Microbac

Accredited Lab Data for Today's Environment

Data Qualifiers

Qualifier	Description
A	E. coli present.
A'	E. coli absent.
B	Analyte detected in associated MB.
C	Sample result confirmed.
D	Results reported from dilution.
E	Analyte concentration exceeds calibration range.
F	Unable to analyze due to sample matrix interference.
H	Sample was received or analyzed past the established holding time.
J	Estimated concentration.
K	Sample contained lighter hydrocarbon fractions.
L	Sample contained heavier hydrocarbon fractions.
M	MS and/or MSD recovery outside acceptance limits.
N	Presumptive evidence of analyte present.
O	Sample hydrocarbon pattern does not match calibration standard pattern.
P	Percent difference between primary and secondary column concentrations exceeds acceptance limit.
Q	LCS outside acceptance limits.
R	Data unusable.
S	Surrogate outside acceptance limits on initial and reanalysis.
S'	Surrogates diluted below detection.
T	Sample received improperly preserved.
U	Analyte not detected.
W	Raised quantitation or reporting limit due to limited sample volume.
Y	Replicate/Duplicate precision outside acceptance limits.
Z'	Calibration criteria exceeded but for this situation acceptable by method.
Z	Calibration criteria exceeded.
M'	Result from Method of Standard Additions (MSA).
Q'	LCS/LCD analyzed due to insufficient sample for MS/MSD.

The uncertainty of analytical results can be calculated using the following equation:

$$n = t*s/1.414$$

where

t=12.706 (Students t value for 95% confidence interval of two replicates)
s= standard deviation of sample and duplicate data
1.414 is square root of the number of replicates (two)

Abbreviations

Laboratory Control Sample	(LCS)
Laboratory Control Duplicate	(LCD)
Matrix Spike	(MS)
Matrix Spike Duplicate	(MSD)
Method Blank	(MB)

APPENDIX J

FIGURE 10:
CHLOROPHYLL a DATASHEET

CHLOROPHYLL-a DATA SHEET
DIX RIVER PROJECT

SAMPLE ID	*COLLECTOR*	*WATERSHED*	*DATE/TIME COLLECTED*	*DATE/TIME FILTERED*	*VOLUME FILTERED*	*TOTAL # FILTER PADS*	*AREA COLLECTED*

Filtering Technician Signature: _____

Form updated 5/10/06 mlw

THIRD ROCK CONSULTANTS

APPENDIX K

TABLE 1:
RESULTS SUMMARY FOR DIX RIVER WATERSHED PROJECT

Table 1: Sample / Results Summary for Dix River Watershed

Parameters	Analyte Name	Clarks Run Select	Clarks Run Non-Select	Hanging Fork Select	Hanging Fork Non-Select	Dix River Select	Dix River Non-Select	TOTAL
Sites	Number of Sites	4	4	6	8	1	8	31
Parameters	Analyte Name	Number of samples*						
Total P	Phosphorus, Total	48	48	60	96	12	96	360
Ortho-P	Phosphorus, Ortho	48	48	60	96	12	96	360
NO2	Nitrite as N	48	48	60	96	12	96	360
NO3	Nitrate as N	48	48	60	96	12	96	360
TKN	Total Kjeldahl Nitrogen	48	48	60	96	12	96	360
NH3-N	Ammonia as N	48	48	60	96	12	96	360
TOC	Organic Carbon, Total	48	48	60	96	12	96	360
TSS	Solids, Total Suspended	48	48	60	96	12	96	360
TC/EColi	Total Coliform / E. coli	48	48	60	96	12	96	360
DO	Dissolved Oxygen	48	48	60	96	12	96	360
Temp	Temperature	48	48	60	96	12	96	360
Cond	Conductivity	48	48	60	96	12	96	360
Flow	Flow	48	48	60	96	12	96	360
pH	pH	48	48	60	96	12	96	360
Turbidity	Turbidity	39	-	42	-	12	-	93
CBOD5	Biochemicial Oxygen Demand, 5-Day Carbonaceous	48	48	60	-	12	-	168
CBOD15	Biochemicial Oxygen Demand, 15-Day Carbonaceous	48	-	-	-	-	-	48
Chlorides	Chloride	16	-	20	-	4	-	40
Chloro a	Chlorophyll a	48	-	60	-	12	-	120
Alkalinity	Alkalinity	48	-	60	-	12	-	120
Periphyton	Periphyon	8	-	12	-	2	-	22
24hr. Diurnal DO	24hr. Diurnal Dissolved Oxygen	2 total from 2 sites						2

***NOTE: Number of samples indicates the expected total number of samples collected at the specified sites over the entire sampling period.**

APPENDIX L

TABLE 2:
METHODS, ANALYTES, AND REPORTING LIMITS FOR THE DIX RIVER WATERSHED

Table 2: Methods, Analytes, and Data Quality Indicators for the Dix River Watershed

Parameters	Analyte Name	Units	Reporting Limit	Precision Criteria (%RPD)	Accuracy Criteria MS (% Uncertainty)	Accuracy Criteria LCS (% Uncertainty)
CBOD15	Biochemicial Oxygen Demand, 15-Day Carbonaceous	mg/L	2	20	N/A	15
CBOD5	Biochemicial Oxygen Demand, 5-Day Carbonaceous	mg/L	2	20	N/A	15
TSS	Solids, Total Suspended	mg/L	3	20	N/A	20
Total P	Phosphorus, Total	mg/L as P	0.4	20	10	10
Ortho-P	Phosphorus, Ortho	mg/L as P	0.14	20	10	10
NO2	Nitrite as N	mg/L as N	0.1	20	20	10
NO3	Nitrate as N	mg/L as N	0.1	20	20	10
NH3-N	Ammonia as N	mg/L as N	0.1	20	10	10
Chlorides	Chloride	mg/L	1	20	20	10
TKN	Total Kjeldahl Nitrogen	mg/L	0.1	20	10	10
TOC	Organic Carbon, Total	mg/L	0.7	20	10	10
Alkalinity	Alkalinity	mg/L CaCO3	7	20	20	20
Turbidity	Turbidity	NTU	0.01	N/A	10	10
pH	pH	S.U.	0-14	N/A	N/A	5
DO	Dissolved Oxygen	mg/L	1	N/A	N/A	10
Temp	Temperature	°F	40	N/A	N/A	5
Cond	Conductivity	umhos/cm	1	N/A	N/A	10
Flow	Flow	ft3/sec	0.33 for small, 0.20 for large	N/A	N/A	N/A
TC/EColi	Total Coliform / E. coli	MPN	0	20	N/A	N/A
Chloro a	Chlorophyll a	ug/L	N/A	20	N/A	10
Periphyton	Periphyon	NA	NA	NA	N/A	NA
24hr. Dinural DO	24hr. Dinural Dissolved Oxygen	mg/L	1	N/A	N/A	15

Definitions:
RPD = Relative Percent Difference
LCS = Laboratory Control Sample
MS= Matrix Spike

APPENDIX M

TABLE 3:
SUMMARY OF PROJECT SAMPLING AND ANALYTICAL REQUIREMENTS

Table 3: Summary of Project Sampling and Analytical Requirements

Parameters	Analyte Name	Method	Minimum Sample Volume	Containers	Preservation	Maximum Hold Time
CBOD15	Biochemicial Oxygen Demand, 15-Day Carbonaceous	EPA 405.1 MOD or SM5210B MOD	1 L	Plastic	Cool 4°C	48 hrs
CBOD5	Biochemicial Oxygen Demand, 5-Day Carbonaceous	EPA 405.1 MOD or SM5210B MOD	1 L	Plastic	Cool 4°C	48 hrs
TSS	Solids, Total Suspended	EPA 160.2	1 L	Plastic	Cool 4°C	7 days
Total P	Phosphorus, Total	EPA 365.1 or 365.4	50mL	Plastic	Cool 4°C, H_2SO_4 to pH <2	28 days
Ortho-P	Phosphorus, Ortho	EPA 300.0 or 365.2	250mL	Plastic	Cool 4°C	48 hrs
NO2	Nitrite as N	EPA 300.0	50ml	Plastic	Cool 4°C	48 hrs*
NO3	Nitrate as N	EPA 300.0	50mL	Plastic	Cool 4°C	48 hrs*
NH3-N	Ammonia as N	EPA 350.1	500mL	Plastic	Cool 4°C, H_2SO_4 to pH <2	28 days
Chloride	Chloride	EPA 300.0	25mL	Plastic	Cool 4°C	28 days
TKN	Total Kjeldahl Nitrogen	EPA 351.2	50mL	Plastic	Cool 4°C, H_2SO_4 to pH <2	28 days
TOC	Organic Carbon, Total	EPA 415.1	25mL	Amber Glass	Cool 4°C, H_2SO_4 to pH <2	28 days
Alkalinity	Alkalinity	EPA 310.1 or 310.2	100mL	Plastic	Cool 4°C	14 days
Turbidity	Turbidity	EPA 180.1	Sufficient volume to submerge probe	Direct source measurement	NA	On-Site [1]
pH	pH	EPA 150.1			NA	Immediately/On-Site
DO	Dissolved Oxygen	EPA 360.1			NA	Immediately/On-Site
Temp	Temperature	EPA 170.1			NA	Immediately/On-Site
Cond	Conductivity	EPA 120.1			NA	On-Site [1]
Flow	Flow	USGS Modified	NA	NA	NA	NA
TC/EColi	Total Coliform / E. coli	SM 9223	100mL	Glass/Plastic, Sterile	Cool <10°C, $Na_2S_2O_3$ (No Cl_2)	24 hrs
Chloro a	Chlorophyll a	SM 10200H**	Varies	Amber Glass	***	****
Periphyton	Periphyton	Douglas, 1958	Varies	Amber Glass	See Note[2]	NA
24hr. Dinural DO	24hr. Dinural Dissolved Oxygen	EPA 360.1	Sufficient volume to submerge probe	Direct source measurement	NA	Immediately/On-Site

* Optional preservation of 250 mL with H2SO4 (1+1) to a pH <2 results in a holdtime of 28 days for Nitrate-Nitrite.

** Trichromatic

*** Cool, 4°C, Protect From Light - Wrap Amber Glass Bottle in Aluminum Foil

**** Concentrate sample as soon as possible after collection. *Filter* samples from waters w/ pH =/> 7.0 can be placed in air tight bag and stored frozen for 3 weeks; *filter* samples from waters w/ pH <7.0 should be processed as soon as possible to prevent chlorophyll degradation.

[1] Samples can be collected for laboratory analysis: Turbidity - 100mls, plastic, cool 4°C, 48hr hold; Conductivity - 100mls, plastic, cool 4°C, 24hr hold if sample is unfiltered/28 day hold if sample is filtered through 0.45um membrane filter.

[2] Lugol's iodine solution, 0.3mL per 100mL of sample

APPENDIX N

TABLE 4:
DIX RIVER WATERSHED ASSESSMENT AND MANAGEMENT REPORTS

Table 4: Dix River Watershed Assessment and Management Reports

Assessment Type	Frequency	Purpose	Internal or External	Parties Responsible for Performing		Method of Reporting
				Performing Assessments	Responding to Assessments	
KDOW Audit	As requested	Ensure conformance to project objectives	External	KDOW	Parties of concern	Corrective Action Response
Laboratory Demonstration of Capability	Prior to initial analysis	Ensure analyst is capable of performing the method to specifications.	Internal	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Laboratory Performance Evaluation	Annually, at minimum	Independent assessment of the accuracy of its analyses	External	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Laboratory Internal Audits	Annually, at minimum	Ensure conformance to methods, regulations, and procedures.	Internal	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Laboratory External Audits	usually biannually	Ensure conformance to methods, regulations, and procedures.	External	Regulatory Body	Laboratory QA Director	Internal Lab documentation
Project Status Meeting	Weekly	Evaluate the status on project related objectives and concerns	Internal	QA Manager	Project Administrator	Status Meeting Minutes
Field Systems Audit	Quarterly, at minimum	Assess sampling technicians adherence to proper documentation and protocols.	Internal	Field Logistics Coordinator	Sampling Technicians	Email Correspondance
Analytical Results Review	Monthly	Assess progress and results of analytical findings of each station.	External	KDOW	Project Administrator	Analytical Monthly Summary

Appendix C – Quality Assurance Evaluation (July 2007)

Quality Assurance Evaluation

Monitoring, Assessment, and TMDL Development for the
Dix River Watershed

for

Kentucky Environmental and Public Protection Cabinet
Department for Environmental Protection
Division of Water
14 Reilly Road
Frankfort, KY 40601

July 2007

THIRD ROCK CONSULTANTS
www.thirdrockconsultants.com
Environmental Analysis & Restoration

Quality Assurance Evaluation

Monitoring, Assessment, and TMDL Development for the
Dix River Watershed

for

Kentucky Environmental and Public Protection Cabinet
Department for Environmental Protection
Division of Water
14 Reilly Road
Frankfort, KY 40601

July 2007

Prepared by:

Steve Evans

Reviewed by:

Marcia Wooton

THIRD ROCK CONSULTANTS
www.thirdrockconsultants.com
Environmental Analysis & Restoration

Table of Contents

Page

TABLE

APPENDICES

I. GENERAL QUALITY CONTROL

During the sample analysis for the Dix River project, the quality of the data was assessed by multiple quality control samples analyzed concurrently with the samples. The *Quality Assurance Project Plan* for the Dix River Watershed provided the guidelines and criteria for the evaluation of data quality to be implemented in all aspects of the project monitoring, assessment, and Total Maximum Daily Load (TMDL) development. What follows is a discussion of the types of quality controls utilized and a discussion of samples that did not meet project requirements.

During the sample analysis performed in Dix River project, the quality of the data was assessed by multiple quality control samples analyzed concurrently with the project samples. The *Quality Assurance Project Plan* for the Dix River Watershed provided the guidelines and criteria for the evaluation of the data quality to be implemented in all aspects of the project monitoring, assessment, and TMDL development.

In general, the quality of the laboratory data generated during this analysis met project criteria. Of the 4,331 analyses performed on samples for this project, 4,157 or 96 percent of all analyses met criteria for all associated quality control samples. The remaining 4 percent exceeded internal laboratory control criteria for one or more quality controls associated with the sample. Appendix A provides a summary of the total number of samples analyzed and those with qualified results for each test category. Appendix B further indicates the type of quality controls that did not meet CT Laboratories' internal control criteria for each test category. Appendix C provides further information including a complete list of all qualified samples and data showing why the sample was qualified.

Although 4 percent of analyses did not meet the laboratory's internal criteria, for many analyses the laboratory's internal quality control was narrower than the project required. Therefore, a much lesser percentage of analyses did not meet project criteria. Some samples did not meet project criteria, but were of sufficient quality for use with specified adjustments to the data. Of all the samples tested, only 18 analyses for Total Kjeldahl Nitrogen were of insufficient quality for analytical use. Thus, 99.6 percent of all laboratory data produced during this project is sufficient for further analytical use.

What follows is a discussion of the types of quality controls utilized and a discussion of samples that did not meet project requirements. Section II of this document discusses the types of quality controls and the calculations that are applicable to these controls. Section III examines the data quality for each test parameter in detail and provides recommendations for use of the data.

II. TYPES OF QUALITY CONTROL

A. Contamination

A method blank (MB) is usually prepared at a frequency of one per 20 field samples. The MB is analyzed at the beginning of every analytical run and prior to the analysis of any samples. MB results are acceptable if the concentrations of the target analyte does not exceed the limit of quantitation or reporting limit (RL). In both COMPASS and laboratory reports, samples associated with a MB exceeding the RL are "B" qualified.

B. Precision

Precision of data is measured in the laboratory by a duplicate or matrix spike duplicate and in the field by a field duplicate or field split.

A duplicate sample (DUP) or duplicate matrix spike sample (MSD) is usually prepared at a frequency of one per 20 field samples. The relative percent difference (RPD) between duplicate samples, for samples having analyte concentrations greater than their respective reporting limit, or between a matrix spike (MS) and matrix spike duplicate (MSD), must be within the acceptance ranges. Samples associated with duplicate samples outside of this range are "Q" qualified in COMPASS and "Y" qualified in laboratory reports.

When calculated for duplicate sample analyses, precision is expressed as the *relative percent difference* (RPD), which is calculated as:

$$RPD\ (\%) = \frac{|S - D|}{(S + D)/2} \times 100$$

where:

S = first sample value (original result)
D = second sample value (duplicate result)

Approximately five percent of all samples taken in the field are duplicated. To perform a field duplicate, the Sampling Technician shall consecutively collect two representative aliquots, independent of one another, from the same source using the same collection technique.

Approximately five percent of all samples taken in the field are split. To perform a field split sample, the Sampling Technician shall evenly divide the contents of one grab collection into two sets of sampling bottles. To ensure the split is representative, sample bottles are each filled in three rounds of filling each bottle one third of the total volume.

C. Accuracy

Laboratories use two types of quality controls to assess the accuracy of data - laboratory control samples and matrix spikes.

A laboratory control sample (LCS) is second-source to the calibration standards and is usually prepared at a frequency of one per every 20 field samples. The LCS results are acceptable if the percent recovery of each analyte is within the determined acceptance range. Samples associated with LCS samples outside of this range are "Q" qualified in COMPASS and laboratory reports.

For LCSs, accuracy is quantified by calculating the *percent recovery* (%R) of analyte from a known quantity of analyte as follows:

$$\%R = \frac{V_m}{V_t} \times 100$$

where:

V_m = measured value (concentration determined by analysis)
V_t = true value (concentration or quantity as calculated or certified by the manufacturer)

A matrix spike (MS) sample or a matrix spike duplicate (MSD) sample is designed to provide information about the effect of the sample matrix on the digestion and measurement methodology. A known amount of the analyte of interest is added to a sample prior to sample preparation and instrumental analysis. Spikes are usually prepared every 20 field samples for each matrix. Spike recoveries must fall within the acceptance ranges. Samples associated with matrix spike or matrix spike duplicate samples outside of this range are "Q" qualified in COMPASS and "M" qualified in laboratory reports.

To assess the effect of sample matrix on accuracy, the %R for the analyte of interest in the spiked sample is calculated as follows:

$$\%R = \frac{(SSR - SR)}{SA} \times 100$$

where:

SSR = spiked sample result
SR = sample result
SA = spike added

D. Other Data Quality Indicators

Three data qualifiers not associated with actual field or laboratory quality controls are provided in COMPASS and laboratory reports for informational purposes. A "U" qualifier indicates that the sample result was lower than the limit of detection for the indicated method and is reported as a less than value. A "J" qualifier indicates that the result is less than the limit of quantification, but greater than the limit of detection for the indicated method. A "J" qualifier provides an indication of the uncertainty associated with reporting within this range. A "D" qualifier indicates that a dilution was performed on the sample as part of the laboratory analysis.

If the sample was not analyzed within the acceptable holding time or if the sample was improperly preserved, a "H" qualifier is applied to laboratory results and a "T" qualifier to COMPASS results.

III. ANALYSIS OF DATA QUALITY

A. Alkalinity

Qualified Samples - 1
% Qualified – 0.7%

The Q qualifier associated with the Chicken Bristle 2/27/07 sampling event was caused by a low recovery on the matrix spike (27%) and matrix spike duplicate (28%). Because laboratory control samples were acceptable for the analysis, the sample results are of acceptable quality for use.

B. Biochemical Oxygen Demand – Carbonaceous, 5-day ($CBOD_5$)

Qualified Samples – 51
% Qualified – 40.2%

Of the 51 Q qualified samples for $CBOD_5$, 4 are due to high RPD values between duplicates and 47 are due to low recovery on the glucose-glutamic acid laboratory control sample.

The Q qualifiers caused by a high RPD on the duplicate are found on samples with results near the quantitation limit, and therefore small differences between results are exaggerated on the percent level. These results are of acceptable quality for use.

The Q qualifiers caused by a low recovery on the laboratory control sample show a low bias in the data. According to Standard Methods, the glucose-glutamic acid (GGA) sample "is intended to be a reference point for the evaluation of dilution water quality, seed effectiveness, and analytical technique." Thus, low recovery in the GGA indicates the presence of toxicants, such as copper, or of relatively inactive microorganism seed, both of which would affect the sample results. Low recovery for GGA samples is common because the quality of the dilution water and the seed material used in the test are variable and difficult to monitor due to the lengthy analysis time of the procedure. Because the hold time (24hr) for $CBOD_5$ is shorter than the analysis time (5 days), laboratory reanalysis of samples with GGA results outside of acceptable limits is impossible.

The laboratory recovery limits for GGA samples are 85-115%. GGA samples were analyzed before and after each set of 20 project samples; if either of these samples fell outside of the recovery limits a qualifier was applied even if the other GGA sample was acceptable. For the 47 samples associated with GGA samples with low recovery, the recovery ranged from 51% to 83%. Most qualified sample results for the Dix River watershed were < 2 mg/L. Adjusting for the sample bias shows that at most the sample results could be < 4 mg/L.

C. Biochemical Oxygen Demand – Carbonaceous, 15-day ($CBOD_{15}$)

Qualified Samples – 1
% Qualified – 3.8%

The Q qualifier associated with the Clark's DOW 7/12/07 sampling event was caused by a high RPD on the duplicate (160%). As this result is near the quantitation limit, small differences between results are exaggerated on the percent level. This result is of acceptable quality for use.

D. Chloride
Qualified Samples – 7
% Qualified –10.1%

Of the 7 chloride samples with qualifiers, 6 matrix spikes and 4 matrix spike duplicates yielded a low recovery and were Q qualified and one sample was analyzed outside of the hold time. All Q qualified results obtained recoveries outside of the 90-110% range established by the laboratory, but were within the 80-120% criteria established for use in this project and are therefore acceptable for use.

One sample at Ball's Branch Mouth on 9/6/07 was analyzed outside of holding time limits.

E. Chlorophyll a
Samples Analyzed - 213

Though no chlorophyll a data is qualified in the laboratory data, 5 sets of field duplicates and field splits results, shown below, show significant variance from the sample results.

TABLE 1 – FIELD QUALITY CONTROL SAMPLES WITH SIGNIFICANT VARIANCE FOR CHLOROPHYLL A

Site Location	Date	Sample Result (mg/m2)	Field Duplicate Result (mg/m2)	Field Split Result (mg/m2)
Chicken Bristle	8/10/2006	792	1448	-
Clark's Run 150	9/05/2006	741.62	493.57	818.76
Dix Above	4/11/2006	64.92	-	125.83
Moore's Lane	4/13/2006	80.60	157.2	-
Moore's Lane	7/07/2006	4108.04	2260.67	-

As field split samples are measured from the same aliquot, variance in these results may be due to heterogeneous divisions of the sediments / algae during the split. Variance in field duplicate results may be due to innate variance in the amount of algae on sampled substrate. An average of the data may be more appropriate for analytical use.

F. Coliform, Total
Samples Analyzed - 415

Although all total coliform samples performed acceptably according to laboratory quality controls, field duplicate and field split results showed large differences that required evaluation. Since Standard Methods does not list criteria to evaluate acceptable recovery of a known sample, an alternate method for establishing acceptable control criteria was utilized in order to evaluate acceptable RPD in field duplicate and field split samples.

The laboratory participates in annual proficiency testing studies as part of its certification requirements. In these studies, statistical analysis is performed on known samples analyzed by multiple labs to establish acceptable recovery criteria. Acceptable criteria for two of these studies were established at 47-211% and 38-261%. Using these studies as a basis for project data analysis, it was assumed that results could acceptably range from 50% to 200% of the reported result. Though this range does not include variability associated with field conditions, this range establishes reasonable criteria for recovery.

With this information, significant variance was defined as a difference between the field sample or field split and the sample result such that the acceptable recovery ranges (50-200%) for each result do not overlap. Using this criteria, two collection events show significant variance; Ball's Branch West 2/27/2007 (24100/100mL vs Split of 646/100mL) and Baughman Creek 9/25/2006 (112350/100mL vs Split of >1209800/100mL). Because of the significant variance in these samples, an average of the data may be more appropriate for analytical use. For the Baughman Creek sample, the upper range value could be utilized in the average.

G. E. coli

Samples Analyzed - 415

Although all E.coli samples performed acceptably according to laboratory quality controls, field duplicate and field split results showed large differences that required evaluation. Since Standard Methods does not list criteria to evaluate acceptable recovery of a known sample, an alternate method for establishing acceptable control criteria was utilized in order to evaluate acceptable RPD in field duplicate and field split samples.

The laboratory participates in annual proficiency testing studies as part of its certification requirements. In these studies, statistical analysis is performed on known samples analyzed by multiple labs to establish acceptable recovery criteria. Acceptable criteria for two of these studies were established at 54-184% and 43-227%. Using these studies as a basis for project data analysis, it was assumed that results could acceptably range from 60% to175% of the reported result. Though this range does not include variability associated with field conditions, this range establishes reasonable criteria for recovery.

With this information, significant variance was defined as a difference between the field sample or field split and the sample result such that the acceptable recovery ranges (60-175%) for each result do not overlap. Using this criteria, three collection events show significant variance; Ball's Branch West 2/27/2007 (4760/100mL vs Split of 20/100mL), Hanging Fork Mouth 9/18/2006 (500/100mL vs Duplicate of 2600/100mL and Split of 1000/100mL), and Oak Creek 8/10/2006 (2100/100mL vs Split of 500/100mL). Because of the significant variance in these samples, an average of the data may be more appropriate for analytical use.

H. Nitrogen, Ammonia

Qualified Samples – 1
% Qualified – 0.3%

The Q qualifier associated with the McCormick Church 7/6/07 sampling event was caused by a low recovery on the matrix spike (75%) and matrix spike duplicate (77%) and a high relative percent difference (RPD) on the duplicate (34%). The result was below the quantitation limit and the method blanks and laboratory control samples were acceptable, so the result is of acceptable quality for use.

I. Nitrogen, Nitrate

Qualified Samples – 11
% Qualified –2.6%

Of the 11 nitrate samples with qualifiers, 9 were "Q" qualified from 8 matrix spikes and 5 matrix spike duplicates with low recoveries and 2 were "T" qualified from analysis outside of hold.

The laboratory acceptance criteria for matrix spikes is 90-110% but the project criteria is 80-120%. Thus, 6 matrix spikes and all matrix spike duplicates meet project criteria despite being outside of the laboratory limits. The remaining two matrix spikes recovered 76% and 66% of the known amount. The associated laboratory control samples were within criteria for these samples and therefore the data is of acceptable quality for use.

Samples from Ball's Branch Mouth 9/6/06 and White Oak 10/4/06 were analyzed outside of the hold time requirements.

One set of field duplicates and field splits results showed large relative percent differences between results. On the 5/8/06 White Oak sampling event, a field duplicate and field split were collected which yielded results of 6.6 mg/L and 0.68 mg/L respectively compared to 9 mg/L for the regular sample. The laboratory quality controls for this analysis were all within acceptance limits indicating that the variation in the results is due to field variability. Results during the year of monitoring at this site yielded results throughout this range indicating that this variability may be inherent to the stream or suspended sediment within the stream. An average of the data may be more appropriate for analytical use.

J. Nitrogen, Nitrite

Qualified Samples – 6
% Qualified –1.4%

Of the 6 nitrite samples with qualifiers, 5 were "Q" qualified from 4 matrix spikes and one matrix spike duplicates with low recovery and one was "T" qualified for analysis outside of the hold time requirements.

The laboratory acceptance criteria for matrix spikes is 90-110% but the project criteria is 80-120%. As all qualified matrix spikes and spike duplicates recovered more than 80% of the expected amount all meet project criteria despite being outside of the laboratory limits and are of acceptable quality for use.

The 9/6/06 sample from Ball's Branch Mouth was analyzed outside of the hold time requirements.

K. Nitrogen, Total Kjeldahl (TKN)
Qualified Samples – 29
% Qualified –7.1%

Of the 29 qualified samples for TKN, 12 were "Q" qualified and 18 "B" qualified due to a method blank result above the limit of quantitation. Of the 12 "Q" qualified samples, 10 were due to duplicates with high RPD, one to a matrix spike with high recovery, and one to a matrix spike duplicate with low recovery and high RPD.

The Q qualifiers caused by a high RPD on the duplicate ranged from 23% to 57% RPD with more than half showing a difference greater than 40%. Because of this large difference, an average of these values may be more acceptable for analytical use.

For the "Q" qualifiers caused by matrix spikes or matrix spike duplicates outside of acceptance criteria, laboratory control samples and the other associated matrix spike control for the sample were within limits. Therefore the data is of acceptable quality for use.

"B" qualifiers were applied to samples potentially affected by laboratory contamination. Positive method blank samples are not uncommon with TKN due to air borne ammonia contamination from other sludges and wastewaters handled in the laboratory. Because contamination could cause false positive, or high biased results between the limit of quantitation and five times the positive blank result, qualifiers are applied to results in this range. All 18 "B" qualified results were collected in November 2006. Because the result could be caused by laboratory contamination, these results are not recommended for analytical use.

L. Organic Carbon, Dissolved (DOC)
Qualified Samples – 3
% Qualified –2.4%

The 3 DOC samples with qualifiers were caused by a high RPD (23%-27%) on the duplicate. The precision criterion is 20% for this project, so these results fall outside of that requirement. In comparison to other DOC results for these stations, both Clark's DOW and Spears are in line with other months, but Mocks is elevated in comparison. Because the duplicate value (8.83 mg/L) for the Mocks's DOC is greater than the reported value (7.0 mg/L) these results are not outliers, but are of acceptable quality for use.

M. Organic Carbon, Total (TOC)
Qualified Samples – 4
% Qualified –1.0%

Of the 4 total TOC samples that are "Q" qualified, 4 are due to a high RPD and one is due to a low matrix spike duplicate recovery.

The three of the four samples qualified for high RPD values were less than 25% RPD. These samples should be of sufficient quality for use. The 1/5/2007 Clark's Run DOW sample has a 88% RPD and

therefore an average of the sample and laboratory duplicate values (1.1 and 2.8 mg/L) may be more appropriate for analytical use.

The qualifier due to the low matrix spike recovery met acceptance criteria for the laboratory control sample and matrix spike duplicate and therefore is of acceptable quality for use.

N. Ortho-Phosphate

Qualified Samples – 19
% Qualified –4.5%

Of the 19 ortho-phosphate samples with qualifiers, 18 were "Q" qualified from 12 matrix spikes and 5 matrix spike duplicates with low recovery and 8 duplicates with high RPDs, and one was "T" qualified for analysis outside of the hold time requirements.

The laboratory acceptance criteria for matrix spikes is 90-110% but the project criteria is 80-120%. Thus, 7 matrix spikes and 4 matrix spike duplicates meet project criteria despite being outside of the laboratory limits. The remaining QC samples ranged from 70% to 79% recovery. The associated laboratory control samples were within criteria for these samples and therefore the data is of acceptable quality for use.

For the 8 duplicates the RPD values ranged from 22% to 44%. The 9/6/06 sample from Ball's Branch Mouth was analyzed outside of the hold time requirements.

O. Phosphorus, Total

Qualified Samples – 38
% Qualified –9.3%

Of the 38 total phosphorus samples that are qualified, 6 are "Q" qualified due to a high RPD and 32 are "B" qualified due to a method blank result above the limit of quantitation.

The laboratory limit of quantitation is 0.01 mg/L, but the project criteria is specified as 0.4 mg/L. All of the "Q" qualified samples and 30 of the 32 "B" qualified samples are below 0.4 mg/L. The two samples with results greater than 0.4 mg/L are greater than five times the positive method blank results; these positives are also less than 0.4 mg/L. If the 0.4 mg/L limit of quantification were utilized, no qualifiers would be necessary for total phosphorus samples. Therefore, all data is acceptable for use, and are only qualified because of the low resolution achieved by the laboratory.

P. Suspended Solids, Total (TSS)

Qualified Samples – 3
% Qualified –0.7%

The 3 TSS samples with qualifiers were caused by a high RPD (38%, 25, 91%) on the duplicate. For each of these samples, the recovery was low (<10mg/L) and therefore differences between results are amplified.

Field duplicate and field split results compared to sample results for most TSS samples. However three sampling events showed significant variance between results; Crab Orchard 7/6/2006 (34.5 mg/L vs. Split of 52.3 mg/L), Oak 8/10/2006 (4 mg/L vs. Split of 32 mg/L), and Moore's Lane 6/6/2006 (170 mg/L vs duplicate of 43.6 mg/L). For each of these values, an average of these results may be more appropriate for analytical use.

Q. Sulfate
Qualified Samples – 2
% Qualified –13.3%

The 2 sulfate samples with "Q" qualifiers both had matrix spikes and matrix spike duplicates with low recovery (72-78%). The associated laboratory control samples were within criteria for these samples and therefore the data is of acceptable quality for use.

R. Sulfide
Qualified Samples – 2
% Qualified –13.3%

Of the 2 sulfide samples with "Q" qualifiers, one had matrix spikes with low recovery (72%) and the other a laboratory control sample with low recovery (72%). An associated quality control (laboratory control sample or matrix spike) with acceptable recovery was analyzed with each of these samples, and therefore the data is of acceptable quality for use.

APPENDICES

APPENDIX A – SUMMARY OF QUALIFIED SAMPLES BY TEST PARAMETER

Summary of Qualified Samples by Test Parameter

Testname	Total Qualifed Samples*^	Total Samples per Parameter	% Qualified Samples
Alkalinity (mg/L)	1	135	0.7%
AGP	0	5	-
Ammonia-Nitrogen (mg/L)	1	407	0.2%
CBOD15 (mg/L)	1	26	3.8%
CBOD5 (mg/L)	51	127	40.2%
Chloride (mg/L)	7	69	10.1%
Chlorophyll A	0	213	-
Dissolved Organic Carbon (mg/L)	3	127	2.4%
E. Coli	0	415	-
LTBOD (BOD90)	0	4	-
Nitrate-Nitrogen (mg/L)	11	425	2.6%
Nitrite-Nitrogen (mg/L)	6	426	1.4%
Ortho-Phosphate (mg/L as P)	19	426	4.5%
Sulfate (mg/L)	2	15	13.3%
Sulfide (mg/L)	2	15	13.3%
Total Coliform	0	415	-
Total Kjeldahl Nitrogen (mg/L)	29	407	7.1%
Total Organic Carbon (mg/L)	4	407	1.0%
Total-Phosphorus (mg/L as P)	38	407	9.3%
TSS	3	407	0.7%
Grand Total	174	4331	4.0%

*Excludes T qualifers for July out of hold samples; includes recollected sample results.
^Samples with more than one qualifier are counted only once in totals.

APPENDIX B – QUALIFIED TEST RESULTS BY QUALITY CONTROL TYPE

Qualified Test Results by Quality Control Type

Count of Type of QC Value	Type of QC Value						
Testname	DUP	LCS	MB	MS	MSD	Out of Hold	Grand Total*^
Ammonia-Nitrogen (mg/L)	1			1	1		3
CBOD15 (mg/L)	1						1
CBOD5 (mg/L)	4	47					51
Chloride (mg/L)				6	4	1	11
Dissolved Organic Carbon (mg/L)	3						3
Nitrate-Nitrogen (mg/L)				8	5	2	15
Nitrite-Nitrogen (mg/L)				4	1	1	6
Ortho-Phosphate (mg/L as P)	8			12	5	1	26
Sulfate (mg/L)				2	2		4
Sulfide (mg/L)		1		1			2
Total Kjeldahl Nitrogen (mg/L)	10		18	1	1		30
Total Organic Carbon (mg/L)	4				1		5
Total-Phosphorus (mg/L as P)	6		32				38
TSS (mg/L)	3						3
Alkalinity (mg/L)				1	1		2
Grand Total*^	40	48	50	36	21	5	200

*Excludes T qualifiers for July out of hold samples; includes recollected sample results.

^Samples with more than one qualifier are counted only once in totals.

APPENDIX C – DETAILED QUALIFIED DATA ANALYSIS

Detailed Qualified Data Analysis

Testname	Flags	Type of QC Value	Sample ID	Result	Result	Unit	Additional Flag Information	Limts	Flag Category
Alkalinity (mg/L)	Q	MS	TRC_CHCKLP01-20070227			mg/l	27%		Acceptable
Alkalinity (mg/L)	Q	MSD	TRC_CHCKLP01-20070227			mg/l	28%	90-110%	Acceptable
Ammonia-Nitrogen (mg/L)	Q	DUP	TRC_MCCCHLP01-20060706	<	0.02	mg/l	34%	20%	Acceptable, <RL
Ammonia-Nitrogen (mg/L)	Q	MS	TRC_MCCCHLP01-20060706	<	0.02	mg/l	75%	90-110%	Acceptable, <RL
Ammonia-Nitrogen (mg/L)	Q	MSD	TRC_MCCCHLP01-20060706	<	0.02	mg/l	77%	90-110%	Acceptable, <RL
CBOD15 (mg/L)	Q	DUP	TRC_CDOWLP01-20060712-RESAMP		2.60	mg/l	160%	20%	
CBOD15 (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD15 (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060712-RESASIMU		2.50	mg/l			T-Reanalyzed
CBOD5 (mg/L)	Q	DUP	TRC_BBMOULP01-20060510			mg/l		20%	
CBOD5 (mg/L)	Q	DUP	TRC_CDOWLP01-20060316			mg/l	50%	20%	
CBOD5 (mg/L)	Q	DUP	TRC_CHCKLP01-20061002			mg/l	74%	20%	
CBOD5 (mg/L)	Q	DUP	TRC_CORPLP01-20070131	<	2.00	mg/l	37%	20%	
CBOD5 (mg/L)	Q	LCS	TRC_BBMOULP01-20060906	<	2.00	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_BBMOULP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_BBMOULP02-20060605	<	2.00	mg/l	58%, 61%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_BBWESTLP01-20061003	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_BBWESTLP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_BBWESTLP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_BBWESTLP01-20061218-DUP	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CDOWLP01-20060605	<	2.00	mg/l	58%, 61%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CDOWLP01-20060906	<	2.00	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CDOWLP01-20061116	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CHCKLP01-20060906	<	2.00	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CHCKLP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CORPLP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CORPLP01-20061113-SPLIT	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CORPLP01-20061218-SPLIT	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR150LP01-20060905-DUP	<	2.00	mg/l	77%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR150LP01-20060905-SPLIT	<	2.00	mg/l	77%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR150LP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR52LP01-20060605	<	2.00	mg/l	58%, 61%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR52LP01-20060906		2.60	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR52LP01-20061003	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR52LP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CR52LP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CRBYLP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_CRBYLP01-20061113-DUP	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_DIXABOVELP01-20060906	<	2.00	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_DIXABOVELP01-20061003	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_DIXABOVELP01-20061115	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_DIXDOWLP01-20060906	<	2.00	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_DIXDOWLP01-20061003	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_DIXDOWLP01-20061116	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_HFMTHLP01-20060907	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_HFMTHLP01-20061003	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_HFMTHLP01-20061128	<	2.00	mg/l	51%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_HFMTHLP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_KNOBLP02-20060907	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_KNOBLP02-20061003	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_KNOBLP02-20061003-DUP	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_KNOBLP02-20061003-SPLIT	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_KNOBLP02-20061128	<	2.00	mg/l	51%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_KNOBLP02-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_MCCCHLP01-20060906	<	2.00	mg/l	83%	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_MCCCHLP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_MOORLP01-20061002	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_MOORLP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_OAKLP01-20061218	<	2.00	mg/l	Low	85-115%	
CBOD5 (mg/L)	Q	LCS	TRC_S2NDLP01-20061113	<	2.00	mg/l	78%	85-115%	
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060712-RESASIMU	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_CORPLP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_CORPLP01-20060712-RESASIMU	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_CRBYLP01-20060707	<	2.00	mg/l			T-Reanalyzed

Detailed Qualified Data Analysis

Testname	Flags	Type of QC Value	Sample ID	Result	Result	Unit	Additional Flag Information	Limts	Flag Category
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_CRBYLP01-20060712-RESASIMU	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_DIXABOVELP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_HFMTHLP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_KNOBLP02-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_MOORLP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_MOORLP01-20060707-DUP	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_OAKLP01-20060707	<	2.00	mg/l			T-Reanalyzed
CBOD5 (mg/L)	T	T-Reanalyzed	TRC_OAKLP01-20060707-SPLIT	<	2.00	mg/l			T-Reanalyzed
Chloride (mg/L)	Q	MS	TRC_CDOWLP01-20060605		36.00	mg/l	87%	90-110%	
Chloride (mg/L)	Q	MS	TRC_CR150LP01-20060905-SPLIT		22.00	mg/l	81%	90-110%	
Chloride (mg/L)	Q	MS	TRC_CR150LP01-20070228		31.00	mg/l	88%	90-110%	
Chloride (mg/L)	Q	MS	TRC_DIXABOVELP01-20070226		8.10	mg/l	89%	90-110%	
Chloride (mg/L)	Q	MS	TRC_HFMTHLP01-20060907		8.40	mg/l	88%	90-110%	
Chloride (mg/L)	Q	MS	TRC_MCCCHLP01-20060906		8.10	mg/l	88%	90-110%	
Chloride (mg/L)	Q	MSD	TRC_CDOWLP01-20060605		36.00	mg/l	88%	90-110%	
Chloride (mg/L)	Q	MSD	TRC_CR150LP01-20060905-SPLIT		22.00	mg/l	80%	90-110%	
Chloride (mg/L)	Q	MSD	TRC_HFMTHLP01-20060907		8.40	mg/l	89%	90-110%	
Chloride (mg/L)	Q	MSD	TRC_MCCCHLP01-20060906		8.10	mg/l	86%	90-110%	
Chloride (mg/L)	T	Out of Hold	TRC_BBMOULP01-20060906			mg/l			
Dissolved Organic Carbon (mg/L)	Q	DUP	TRC_CDOWLP01-20061218		3.20	mg/l	27%	20%	
Dissolved Organic Carbon (mg/L)	Q	DUP	TRC_MOCKSLP03-20060706		7.00	mg/l	23%	20%	
Dissolved Organic Carbon (mg/L)	Q	DUP	TRC_SPEARSLP02-20060808		2.20	mg/l	24%	20%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_CR52LP01-20070131		6.90	mg/l	76%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_CRBYLP01-20060707		1.80	mg/l	82%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_DIXABOVELP01-20060714-RESAMP		0.76	mg/l	82%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_DIXDOWLP01-20060706		0.53	mg/l	83%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_HFMTHLP01-20060810		0.22	mg/l	86%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_MCCCHLP01-20060809		0.21	mg/l	88%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_S2NDLP01-20060802		1.10	mg/l	66%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MS	TRC_WHITEOAKLP01-20070130-SPLIT		2.10	mg/l	84%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MSD	TRC_CRBYLP01-20060707		1.80	mg/l	83%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MSD	TRC_DIXABOVELP01-20060714-RESAMP		0.76	mg/l	82%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MSD	TRC_DIXDOWLP01-20060706		0.53	mg/l	82%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MSD	TRC_LOGANLP01-20060803-SPLIT		10.00	mg/l	89%	90-110%	
Nitrate-Nitrogen (mg/L)	Q	MSD	TRC_MCCCHLP01-20060809		0.21	mg/l	88%	90-110%	
Nitrate-Nitrogen (mg/L)	T	Out of Hold	TRC_BBMOULP01-20060906			mg/l			
Nitrate-Nitrogen (mg/L)	T	Out of Hold	TRC_WHITEOAKLP01-20061004		9.90	mg/l			
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_DRAKESLP01-20060707		1.20	mg/l			
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060707		4.70	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060712-RESASIMU		4.20	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CORPLP01-20060707		1.80	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CORPLP01-20060712-RESASIMU		0.20	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CRBYLP01-20060707		1.80	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CRBYLP01-20060712-RESASIMU		0.71	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_DIXABOVELP01-20060707		0.72	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_GILBLP01-20060707		0.07	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_HF150LP01-20060707		2.20	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_HFMTHLP01-20060707		2.30	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_JUNCTLP01-20060707		0.25	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_KNOBLP02-20060707		1.10	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_LOGANLP01-20060707		2.30	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_MOORLP01-20060707		1.50	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_MOORLP01-20060707-DUP		1.50	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_OAKLP01-20060707		0.30	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_OAKLP01-20060707-SPLIT		0.31	mg/l			T-Reanalyzed
Nitrate-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_WHITEOAKLP01-20060707		2.50	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	Q	MS	TRC_CR150LP01-20070228	<	0.07	mg/l	89%	90-110%	
Nitrite-Nitrogen (mg/L)	Q	MS	TRC_CR52LP01-20070131	<	0.07	mg/l	88%	90-110%	
Nitrite-Nitrogen (mg/L)	Q	MS	TRC_CRBYLP01-20060712-RESAMP	<	0.07	mg/l	88%	90-110%	
Nitrite-Nitrogen (mg/L)	Q	MS	TRC_JUNCTLP01-20061127-DUP	<	0.07	mg/l	88%	90-110%	
Nitrite-Nitrogen (mg/L)	Q	MSD	TRC_CRBYLP01-20060712-RESAMP	<	0.07	mg/l	88%	90-110%	
Nitrite-Nitrogen (mg/L)	T	Out of Hold	TRC_BBMOULP01-20060906			mg/l			
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CDOWLP01-20060712-RESASIMU		0.13	mg/l			T-Reanalyzed

Detailed Qualified Data Analysis

Testname	Flags	Type of QC Value	Sample ID	Result	Result	Unit	Additional Flag Information	Limts	Flag Category
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CORPLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CORPLP01-20060712-RESASIMU	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CRBYLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_CRBYLP01-20060712-RESASIMU	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_DIXABOVELP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_DRAKESLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_GILBLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_HF150LP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_HFMTHLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_JUNCTLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_KNOBLP02-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_LOGANLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_MOORLP01-20060707		0.09	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_MOORLP01-20060707-DUP		0.08	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_OAKLP01-20060707	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_OAKLP01-20060707-SPLIT	<	0.07	mg/l			T-Reanalyzed
Nitrite-Nitrogen (mg/L)	T	T-Reanalyzed	TRC_WHITEOAKLP01-20060707		0.29	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_BLUELP01-20061002		0.10	mg/l	37%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_DIXABOVELP01-20060714-RESAMP		0.12	mg/l	32%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_DIXDOWLP01-20061116		0.14	mg/l	25%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_HFMTHLP01-20060907		0.11	mg/l	22%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_HFMTHLP01-20061003		0.13	mg/l	44%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_KNOBLP02-20070105		0.06	mg/l	24%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_MCCCHLP01-20060906			mg/l	30%	20%	
Ortho-Phosphate (mg/L as P)	Q	DUP	TRC_PEYTLP01-20060906		0.08	mg/l	36%	20%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_BBWESTLP01-20070227-SPLIT		0.07	mg/l	77%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_BLUELP01-20061002		0.10	mg/l	118%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_CR150LP01-20060905-SPLIT		0.17	mg/l	112%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_CR150LP01-20070228		0.05	mg/l	76%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_CR52LP01-20070131		0.29	mg/l	70%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_CRBYLP01-20060707		0.14	mg/l	82%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_DIXABOVELP01-20060714-RESAMP		0.12	mg/l	88%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_DIXABOVELP01-20070226	<	0.01	mg/l	86%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_DIXDOWLP01-20061116		0.14	mg/l	82%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_MCCCHLP01-20060906			mg/l	111%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_S2NDLP01-20060802		0.22	mg/l	74%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MS	TRC_WHITEOAKLP01-20070130-SPLIT		1.00	mg/l	76%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MSD	TRC_CR150LP01-20060905-SPLIT		0.17	mg/l	114%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MSD	TRC_CRBYLP01-20060707		0.14	mg/l	87%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MSD	TRC_DIXABOVELP01-20060714-RESAMP		0.12	mg/l	88%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MSD	TRC_MCCCHLP01-20060906			mg/l	112%	90-110%	
Ortho-Phosphate (mg/L as P)	Q	MSD	TRC_S2NDLP01-20060802		0.22	mg/l	79%	90-110%	
Ortho-Phosphate (mg/L as P)	T	Out of Hold	TRC_BBMOULP01-20060906			mg/l			
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_CDOWLP01-20060707		0.28	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_CDOWLP01-20060712-RESASIMU		0.20	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_CORPLP01-20060707		0.09	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_CORPLP01-20060712-RESASIMU		0.06	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_CRBYLP01-20060707		0.14	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_CRBYLP01-20060712-RESASIMU		0.11	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_DIXABOVELP01-20060707		0.05	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_DRAKESLP01-20060707	<	0.01	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_GILBLP01-20060707		0.05	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_HF150LP01-20060707		0.10	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_HFMTHLP01-20060707		0.12	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_JUNCTLP01-20060707	<	0.01	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_LOGANLP01-20060707		0.10	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_MOORLP01-20060707	<	0.01	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_MOORLP01-20060707-DUP	<	0.01	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_OAKLP01-20060707-SPLIT	<	0.01	mg/l			T-Reanalyzed
Ortho-Phosphate (mg/L as P)	T	T-Reanalyzed	TRC_WHITEOAKLP01-20060707		1.10	mg/l			T-Reanalyzed
Sulfate (mg/L)	Q	MS	TRC_CDOWLP01-20060605		41.00	mg/l	78%	90-110%	
Sulfate (mg/L)	Q	MS	TRC_DIXDOWLP01-20060706		9.50	mg/l	76%	90-110%	
Sulfate (mg/L)	Q	MSD	TRC_CDOWLP01-20060605		41.00	mg/l	78%	90-110%	
Sulfate (mg/L)	Q	MSD	TRC_DIXDOWLP01-20060706		9.50	mg/l	76%	90-110%	

Detailed Qualified Data Analysis

Testname	Flags	Type of QC Value	Sample ID	Result	Result	Unit	Additional Flag Information	Limits	Flag Category
Sulfide (mg/L)	Q	LCS	TRC_CDOWLP01-20060707	<	1.00	mg/l	72%	80-120%	
Sulfide (mg/L)	Q	MS	TRC_DIXDOWLP01-20060706	<	1.00	mg/l	72%	80-120%	
Sulfide (mg/L)	T	T-Reanalyzed	TRC_DIXDOWLP01-20060706	<	1.00	mg/l			T-Reanalyzed
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_BAUGHLP01-20061127		0.43	mg/l	23%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_BAUGHLP01-20061218		0.38	mg/l	31%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_CDOWLP01-20060605		1.20	mg/l	49%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_CORPLP01-20061002		0.26	mg/l	38%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_FROGLP01-20070130		0.13	mg/l	57%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_GUMLP01-20061219		0.26	mg/l	26%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_LOGANLP01-20070227		0.55	mg/l	25%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_MOORLP01-20061002		0.59	mg/l	47%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_MOORLP01-20061002			mg/l	47%	20%	
Total Kjeldahl Nitrogen (mg/L)	Q	DUP	TRC_PEYTLP01-20070226		0.25	mg/l	43%	20%	
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_BAUGHLP01-20061127		0.43	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_BLUELP01-20061127		0.47	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_CDOWLP01-20061116		1.40	mg/l	0.552	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_CHCKLP01-20061127		0.40	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_DIXDOWLP01-20061116		0.85	mg/l	0.552	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_FROGLP01-20061127		1.20	mg/l	0.115	0.1	Acceptable
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_HF150LP01-20061128		0.41	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_HFMTHLP01-20061128		0.36	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_JUNCTLP01-20061127		0.32	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_JUNCTLP01-20061127-DUP		0.31	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_MCCCHLP01-20061127		0.46	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_MCCCHLP01-20061127-SPLIT		0.41	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_MCKINLP01-20061127		0.49	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_MOORLP01-20061127		0.37	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_OAKLP01-20060810-SPLIT		0.32	mg/l	0.163	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_OAKLP01-20061127	<	0.10	mg/l	0.115	0.1	Acceptable
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_PEYTLP01-20061127		0.52	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	B	MB	TRC_WHUSTLP01-20061127		0.23	mg/l	0.115	0.1	<10X MB
Total Kjeldahl Nitrogen (mg/L)	Q	MS	TRC_GILBLP01-20060605		0.53	mg/l	121%	80-120%	
Total Kjeldahl Nitrogen (mg/L)	Q	MSD	TRC_CHCKLP01-20060906			mg/l	66%, 39%	90-110%, 20%	
Total Organic Carbon (mg/L)	Q	DUP	TRC_CDOWLP01-20070105		1.10	mg/l	88%	20%	
Total Organic Carbon (mg/L)	Q	DUP	TRC_HFMTHLP01-20060907		1.60	mg/l	21%	20%	
Total Organic Carbon (mg/L)	Q	DUP	TRC_MCKINLP01-20061218		0.80	mg/l	21%	20%	
Total Organic Carbon (mg/L)	Q	DUP	TRC_MOORLP01-20060905		1.10	mg/l	25%	20%	
Total Organic Carbon (mg/L)	Q	MSD	TRC_MCKINLP01-20061218		0.80	mg/l	55%	90-110%	
Total-Phosphorus (mg/L as P)	Q	DUP	TRC_CORPLP01-20061002		0.08	mg/l	28%	20%	
Total-Phosphorus (mg/L as P)	Q	DUP	TRC_KNOBLP02-20070105		0.28	mg/l	117%	20%	
Total-Phosphorus (mg/L as P)	Q	DUP	TRC_LOGANLP01-20061003		0.04	mg/l	27%	20%	
Total-Phosphorus (mg/L as P)	Q	DUP	TRC_LOGANLP01-20070227		0.01	mg/l	418%	20%	
Total-Phosphorus (mg/L as P)	Q	DUP	TRC_MCCCHLP01-20061002		0.07	mg/l	28%	20%	
Total-Phosphorus (mg/L as P)	Q	DUP	TRC_MOORLP01-20061002		0.02	mg/l	9133%	20%	
Total-Phosphorus (mg/L as P)	B	MB	TRC_BAUGHLP01-20060605		0.07	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_BBMOULP02-20060605		0.10	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_BBWESTLP01-20060606		0.08	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_BLUELP01-20060605		0.11	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_CDOWLP01-20060605		0.23	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_CHCKLP01-20060606		0.10	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_COPPLP01-20060605		0.06	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_CORPLP01-20060606		0.09	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_CR150LP01-20060606		0.15	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_CR52LP01-20060605		0.45	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_CRABLP01-20060605		0.10	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_DIXABOVELP01-20060606		0.12	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_DIXDOWLP01-20060606		0.11	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_DIXDOWLP01-20060606-DUP		0.11	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_DRAKESLP01-20060605		0.08	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_FROGLP01-20060605		0.08	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_GILBLP01-20060605		0.05	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_GUMLP01-20060605		0.05	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_HF150LP01-20060607		0.10	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_HFMTHLP01-20060607		0.09	mg/l	0.0181 & 0.0533	0.01	<10X MB

Detailed Qualified Data Analysis

Testname	Flags	Type of QC Value	Sample ID	Result	Result	Unit	Additional Flag Information	Limts	Flag Category
Total-Phosphorus (mg/L as P)	B	MB	TRC_HFMTHLP01-20060607-SPLIT		0.11	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_JUNCTLP01-20060605		0.05	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_KNOBLP02-20060606		0.08	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_LOGANLP01-20060605		0.42	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_MCCCHLP01-20060606		0.11	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_MCKINLP01-20060605		0.11	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_MOORLP01-20060606		0.05	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_MOORLP01-20060606-DUP		0.09	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_OAKLP01-20060606		0.05	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_PEYTLP01-20060605		0.13	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_S2NDLP01-20060606		0.09	mg/l	0.0181 & 0.0533	0.01	<10X MB
Total-Phosphorus (mg/L as P)	B	MB	TRC_WHUSTLP01-20060605		0.06	mg/l	0.0181 & 0.0533	0.01	<10X MB
TSS (mg/L)	Q	DUP	TRC_CR150LP01-20061002			mg/l	38%	20%	
TSS (mg/L)	Q	DUP	TRC_DIXABOVELP01-20061219			mg/l	25%	20%	
TSS (mg/L)	Q	DUP	TRC_HFMTHLP01-20060810		3.40	mg/l	91%	20%	

Appendix D – Quality Assurance Project Plan: Microbial Source Tracking in the Dix River Watershed (May 2008)

Quality Assurance Project Plan

Microbial Source Tracking in the Dix River Watershed

Prepared for
Kentucky Environmental and Public Protection Cabinet
July 23, 2007
Revised May 7, 2008

Prepared by
Third Rock Consultants, LLC
2526 Regency Road
Lexington, KY 40503
859.977.2000
www.thirdrockconsultants.com

THIRD ROCK CONSULTANTS

Quality Assurance Project Plan

Microbial Source Tracking in the Dix River Watershed

for

Kentucky Environmental and Public Protection Cabinet
Department for Environmental Protection
Division of Water
14 Reilly Road
Frankfort, KY 40601

July 23, 2007
Revised May 7, 2008

THIRD ROCK CONSULTANTS
www.thirdrockconsultants.com
Environmental Analysis & Restoration

Distribution and Review List

Quality Assurance Program Plan for Dix River Watershed
Revision: 2, Dated: May 7, 2008

1) Third Rock Consultants, LLC

Project Administrator

Tony Miller — May 7, 2008 — Date

Quality Assurance Manager

Steve Evans — May 7, 2008 — Date

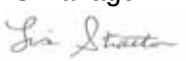
Sampling and Laboratory Coordinator

Marcia Wooton — May 7, 2008 — Date

Data Manager

Jason Remley — May 7, 2008 — Date

GIS Manager



Lisa Stratton — May 7, 2008 — Date

2) Source Molecular

Laboratory Director

Thierry Tamers — Date

Quality Manager

Dr. Troy Scott, PhD — Date

Table of Contents

FIGURE

TABLES

EXHIBITS

APPENDICES

1 Project Management

1.1 Introduction

The Kentucky Division of Water (KDOW) approved this Quality Assurance Project Plan (QAPP) prepared by Third Rock Consultants, LLC (Third Rock). This QAPP covers the planning, implementation, and assessment procedures necessary to meet the minimum data quality objectives (DQOs) while tracking the sources of microbial pollution in the Dix River Watershed, Kentucky.

Third Rock is committed to producing quality data that will assist the Division of Water in the development of their watershed plan. This QAPP is designed to provide a complete plan for achieving all project data quality objectives. However, effective communication is required to ensure all parties properly implement the plan. Any quality feedback, questions, or concerns related to the project should be communicated to the project administrator or quality manager to facilitate appropriate analysis and resolution.

1.2 Project Organization

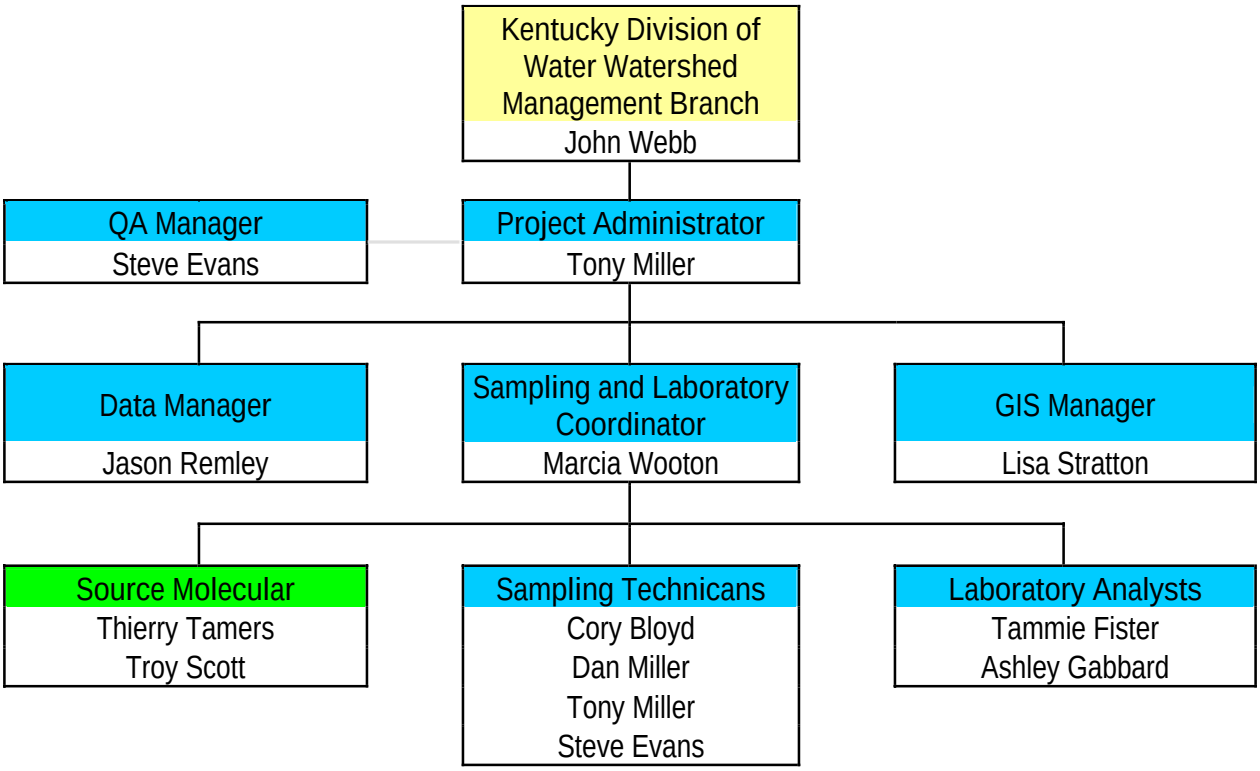
1.2.1 Kentucky Division of Water, Primary Data User

The microbial source tracking analysis conducted by Third Rock for the Dix River Watershed will be under the jurisdiction and oversight of the Kentucky Division of Water (KDOW) Watershed Management Branch. John Webb serves as the KDOW Project Manager, providing overall direction and guidance to the project. Third Rock's project administrator will communicate directly with Mr. Webb to ensure that all project objectives are satisfied.

1.2.2 Third Rock Personnel and QA Responsibilities

The implementation of the project plan requires effective operation of the project team under the management of the KDOW Project Manager. Figure 1, page 6, identifies the parties that comprise the Dix River Project Team and the lines of authority and communication under which this team operates. The solid lines indicate lines of authority both within the Third Rock team (in blue) and with external organizations. The dashed line indicates independent review and oversight. The specific roles and responsibilities of each key party are documented below.

FIGURE 1 - DIX RIVER ORGANIZATIONAL CHART



- *Project Administrator*

Tony Miller will serve as the Project Administrator. Mr. Miller is responsible for the overall completion of the project to the requirements of the KDOW. In this capacity, he is responsible for overall project administration, personnel, scheduling, and completion of all data quality objectives. Additionally, he maintains project financials and contracts and submits reports to the KDOW. Mr. Miller serves as the primary contact with the Kentucky Division of Water.

- *Quality Assurance Manager*

Steve Evans will serve as the Quality Assurance Manager. Mr. Evans is responsible for writing, maintenance, and review of the QAPP, as well as the review of field operations procedures, laboratory procedures, and data documentation that will help ensure field and laboratory data generated meet data quality objectives. Mr. Evans will ensure that the project team is properly trained in the techniques utilized, and that the proper methodology is followed. When involved in the data collection effort, Mr. Evans will have independent oversight and review, but will remain otherwise independent. He is responsible for the maintenance and distribution of the approved QAPP.

- *Sampling and Laboratory Coordinator*

Marcia Wooton will serve as the Sampling Coordinator. Ms. Wooton is responsible for the coordination of sampling events and all aspects of laboratory service.

As Sampling Coordinator, Ms. Wooton is responsible for preparing field collection kits and ensuring that sampling procedures and schedules are implemented by the Sampling Technicians. She also reviews sampling

documentation for accuracy and completeness. In addition, she communicates with the laboratories during each sampling event to ensure that hold-time requirements and other data quality objectives are met.

As Laboratory Coordinator, Ms. Wooton is responsible for the supervision of all functional aspects of Third Rock's laboratory. She is responsible for ensuring that laboratory analysts implement established internal QA/QC procedures and comply with applicable regulations and methodology. Ms. Wooton is also responsible for direct communications with the subcontract laboratory (Source Molecular) regarding sampling event scheduling, sample shipments, and required analysis/reporting. In this capacity, Ms. Wooton also provides preliminary analytical data review and approval.

- *Data Manager*

Jason Remley will serve as the Data Manager. Mr. Remley is responsible for ensuring all laboratory and field data is correctly entered into the COMPASS access database. He ensures that data is correctly formatted according to KDOW requirements, and manages the submission of the electronic database files. Mr. Remley maintains Third Rock's custom designed COMPASS verification program and data entry tool allowing for streamlined data entry as well as verified accuracy with verification reports.

- *GIS Manager*

Lisa Stratton will serve as the GIS Manager. Ms. Stratton will gather household information from the area county health departments for compilation into a GIS data layer shape file. She will ensure that all the necessary metadata is collected and the GIS mapping meets project requirements.

1.2.3 Source Molecular Corporation

The analytical subcontractors for the microbial source tracking laboratory analysis for this project will be Source Molecular Corporation (Source Molecular). The laboratory will be responsible for analysis of samples delivered such that data quality objectives are met. The laboratory will implement and document QA/QC activities to support the results of the analyses performed on the samples. All analyses are expected to be conducted in accordance with the specified analytical methods, the laboratory's Quality Policy Manual (2005), and this QAPP.

The following provides a general summary of the QA responsibilities of key laboratory personnel at Source Molecular:

- *Laboratory Director and Quality Manager*

Thierry Sam Tamers and Troy M. Scott, PhD will jointly serve as Laboratory Directors. In addition, Mr. Scott will serve as the Quality Manager for Source Molecular on this project.

Mr. Tamers routinely serves as Chief Operating Officer of the Corporation and his responsibilities include client interaction, reporting, and quality control of all released documents.

Troy M. Scott, Ph.D. routinely serves as Laboratory Director/Quality Manager. As such his responsibilities include oversight and validation of final results, implementation and enforcement of all quality assurance/quality control measures, supervision of sample processing, and direction of the environmental parasitology and molecular biology divisions of the company. Mr. Scott also maintains the current laboratory Quality Manual, oversees and validates

final results, prepares final reports, supervises analyses, serves as director of Research and Development, heads the Tissue Cell Culture Laboratory, and directs the Environmental Virology Laboratory.

1.3 Problem Definition and Background

Herrington Lake, in the Kentucky River Basin, was formed by the impoundment of the Dix River. As is common with many reservoirs, Herrington Lake is subject to excessive nutrient loading resulting from point and nonpoint source contributions within the watershed. The Dix River Watershed has 24 permitted wastewater-discharge sites and Herrington Lake directly receives wastewater from 6 of the 24 wastewater-discharge sites. In addition, the Dix River Watershed contains failing septic systems, agricultural activities including numerous cattle with free access to streams, and development/construction activities. This abundant nutrient input has lead to the deterioration of water quality, problematic algal blooms, and subsequent fish kills.

Herrington Lake was listed in the 2004 303(d) report as a 1st priority impaired waterbody for aquatic life (non-support) and fish consumption (partial support). The major tributaries to the reservoir, Dix River, Clarks Run, and Hanging Fork, were also cited in the 2004 303(d) report as having segments listed as 1st priority impaired in regards to aquatic life support and primary contact (non-support and partial support). The cited reasons for impairment are primarily low levels of dissolved oxygen (DO) and high levels of bacteria. Sources of both impairments stem from agricultural runoff, septic tank leakage, urban/suburban stormwater runoff, and wastewater treatment plant (WWTP) discharges (USGS 2000).

As part of KDOW's 1998 Clean Water Action Plan, the Natural Resources Conservation Service (NRCS) and KDOW jointly selected five priority watersheds in Kentucky for targeted water quality improvement. The Dix River was selected as one of these priority watersheds. Since that time, several groups have performed monitoring and analysis towards remediation of the Dix River Watershed to a fully supporting status.

In 2005, a monitoring analysis of Peyton Creek and Frog Branch, two tributaries of Hanging Fork, was conducted by Cumberland Environmental Group under the direction of the Heritage RC&D Council (2005). Results showed that Peyton Branch was more severely impacted than Frog Branch.

Currently, the KDOW is in the process of developing a Total Maximum Daily Load (TMDL) and watershed plan for the Dix River including Clarks Run, Hanging Fork, and Herrington Lake. A TMDL identifies pollutant sources and the amount of pollutants from each source, and makes recommendations for pollutant loads a stream can handle without violating water quality standards. The watershed plan is "a means to resolve and prevent water quality problems that result from both point source and nonpoint source problems" (USEPA 2005a).

In 2007, Third Rock completed a modeling study to identify nutrient and pathogen levels throughout the Dix River watershed in support of a TMDL for nutrients and dissolved oxygen for Clarks Run. Additionally, KDOW plans to calculate a TMDL for pathogens for Hanging Fork from data provided by the Third Rock sampling effort.

A key component in the completion of a TMDL is the identification of the source of the pollutant. Although the loading of bacterial inputs in the Hanging Fork watershed has been characterized by monitoring, the identification of the source of the inputs has yet to be identified. Methodologies for identifying the source of all bacterial or microbial contamination are categorized as Microbial Source Tracking (MST).

Under the funding of a Section 319(h) Nonpoint Source Grant, the KDOW has selected a large portion of Hanging Fork Watershed and Balls Branch of the Clarks Run watershed for MST. Within the Hanging Fork Watershed, MST will be conducted at multiple sites located on Hanging Fork and its tributaries upstream of the previously established McCormick Church and Blue Lick monitoring sites. Within this area, the project objectives include:

- Identification of the geographic location of bacterial input sources upstream of specified locations,
- Calculation of the relative contributions of pollutant sources by category, and
- Development of a GIS data set for human wastewater sources including treatment type and location for Lincoln and Boyle Counties

1.4 Project Description

1.4.1 Summary

Third Rock's ultimate goal mirrors that of the KDOW: to remove the tributaries upstream of Herrington Lake (and ultimately Herrington Lake) from the 303(d) list of impaired streams by providing information that will focus water quality improvement actions. Specifically for this project, our goal is to identify and quantify the sources of pathogen pollution to facilitate effective remediation.

In order to accomplish this goal, specific project tasks of Third Rock are as follows:

1. Identify sites for monitoring on the Dix River Watershed and characterize the site for potential pathogen pollution sources.
2. Develop a GIS data set of human wastewater sources in Lincoln and Boyle Counties from the public health department records indicating source types and locations.
3. Perform monitoring and laboratory analyses to identify "hotspot" sites of highest *E. coli* levels and a general indication of the source using the ratio of total coliform atypical colonies to typical colonies (AC/TC).
4. Utilize polymerase chain reaction (PCR) and quantitative PCR (qPCR) methods to identify and quantify the sources.
5. Prioritize sources of impairments and recommend remediation measures in a final report.

Achievement of these objects is expected to occur as scheduled in Table 1, page 10. In general, the investigation will last 6 to 12 calendar months from start to report preparation. For each of the goals specified above, a summary of the tasks associated with accomplishing each goal is presented in more detail in the following sections.

TABLE 1 – DIX RIVER PROJECT SCHEDULE

EVENT	PROJECT SCHEDULE
1. Perform data review	1 month
2. Develop QAPP (with review and revision)	1 month
3. Conduct final site characterization, screening, selection	1 month
4. Conduct monitoring and laboratory analysis	2-4 months*
5. Conduct results analysis	5 months
6. Conduct briefing meetings	Quarterly, or as needed
7. Prepare and submit final report	6 months

*Sampling schedule may be adjusted to account for seasonality.

1.4.2 Development of GIS Set of Human Wastewater Sources

GIS data set development for human wastewater sources will be conducted for Boyle and Lincoln Counties. At the Boyle and Lincoln County Health Departments, information on all residences and businesses will be compiled into a database to be plotted on a GIS map.

The GIS Manager will oversee the conversion of the county health departments' hardcopy files into an electronic database including the type and age of wastewater treatment system, type of facility, location, and any general notes on the condition of the system. The GIS Manager will utilize the locations in this database to construct GIS shape files. The construction of this database is recommended to occur concurrent with site identification and characterization, but may occur afterward.

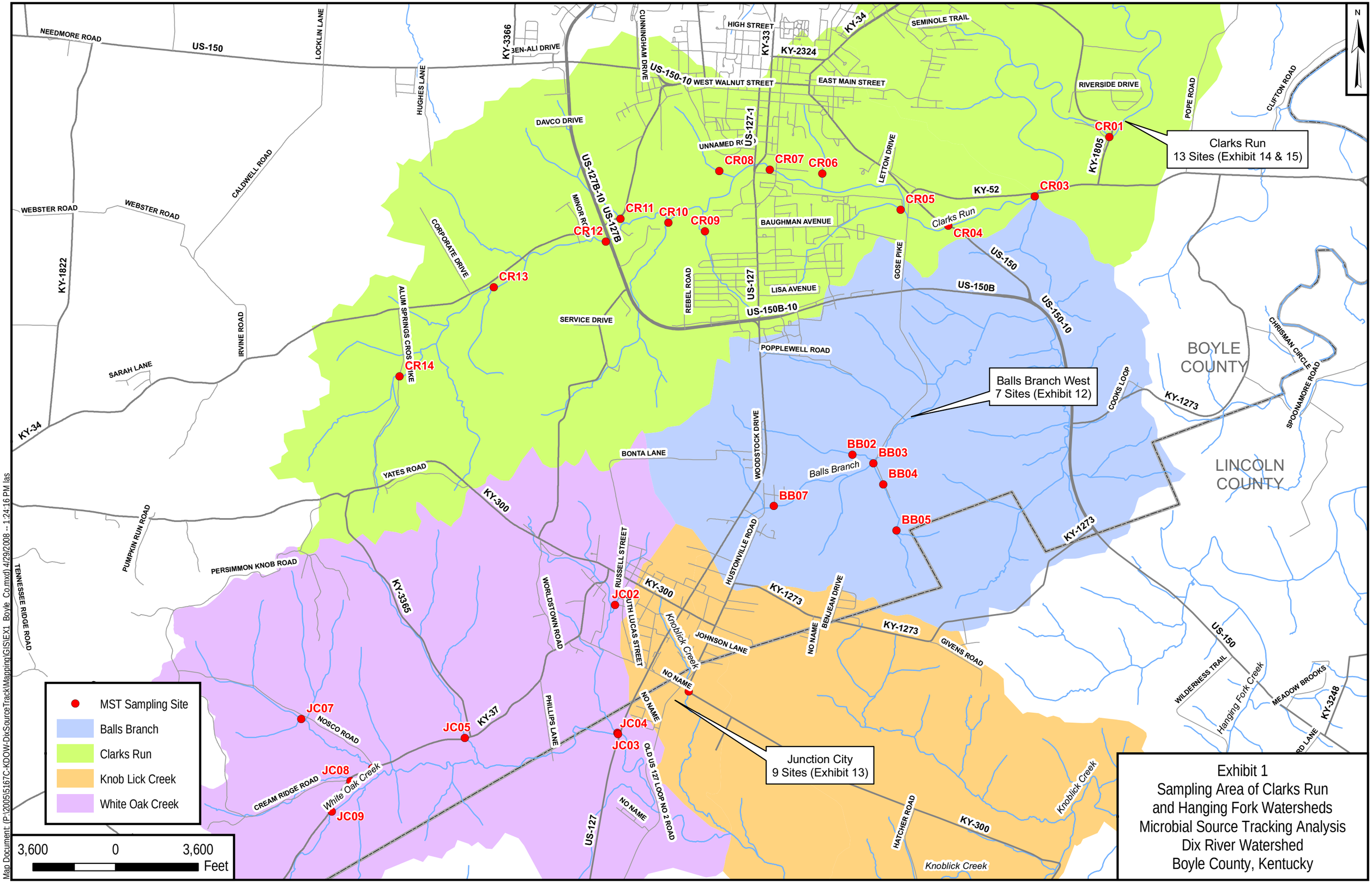
1.4.3 Site Identification and Characterization

Project aerials were surveyed for a combination of variables including land use, site access, existing data implications, and potential source contributions to establish potential sites for characterization and analysis. Using these variables, 74 sites are proposed for analysis in this project as seen in Exhibits 1 and 2, pages 11 and 12. These sites locations are divided according to their location along logical divisions of the watershed. Each of these potential site locations will be visually surveyed by the Sampling Technicians to ensure the optimal location of the final site locations. Once the final site locations are established, site characterization will be performed to assess potential sources of pollution in the area.

During the time period of site characterization and identification, reference samples will be collected and sent to Source Molecular for analysis.

1.4.4 E.coli and Total Coliform Analysis for Hotspot Identification

The Sampling and Laboratory Coordinator will schedule the collection and analysis of grab samples for *E. coli* and total coliform (atypical and typical colonies) at each of the 74 site locations during the Primary Contact Recreation Season (May through October). Two sampling events will be scheduled for this testing: a dry event and a wet event. Third Rock laboratory analysts will perform the *E. coli* and total coliform analysis on all samples.


NEEDMORE ROAD
US-150
LOCKLIN LANE
KY-3366
HUGHES LANE
BEN-ALI DRIVE
CUNNINGHAM DRIVE
US-150-10 WEST WALNUT STREET
HIGH STREET
KY-33
KY-2324
KY-34
SEMINOLE TRAIL
EAST MAIN STREET
RIVERSIDE DRIVE
POPE ROAD
CLIFTON ROAD
DAVCO DRIVE
US-127-1
UNNAMED RD
CR01
KY-1805
Clarks Run
13 Sites (Exhibit 14 & 15)
CR08
CR07
CR06
LETTON DRIVE
US-127B-10
US-127B
CR11
CR10
CR09
CR12
MINOR RD
BAUGHMAN AVENUE
KY-52
CR03
CR05
Clarks Run
CR04
WEBSTER ROAD
WEBSTER ROAD
CALDWELL ROAD
KY-1822
CORPORATE DRIVE
CR13
ALUM SPRINGS CROSS PIKE
REBEL ROAD
US-127
LISA AVENUE
GOSE PIKE
US-150
US-150B
US-150B-10
US-150-10
SERVICE DRIVE
POPPLEWELL ROAD
IRVINE ROAD
SARAH LANE
CR14
KY-34
Balls Branch West
7 Sites (Exhibit 12)
COOKS LOOP
KY-1273
BOYLE COUNTY
CHRISMAN CIRCLE
SPOONAMORE ROAD
BONTA LANE
WOODSTOCK DRIVE
BB02
BB03
BB04
Balls Branch
BB07
BB05
LINCOLN COUNTY
KY-1273
YATES ROAD
KY-300
PUMPKIN RUN ROAD
PERSIMMON KNOB ROAD
TENNESSEE RIDGE ROAD
KY-3365
RUSSELL STREET
WORLDSTOWN ROAD
JC02
SOUTH LUCAS STREET
HUSTONVILLE ROAD
KY-300
KY-1273
BENJEAN DRIVE
KY-1273
JOHNSON LANE
Knoblick Creek
NO NAME
NO NAME
GIVENS ROAD
US-150
WILDERNESS TRAIL
Hanging Fork Creek
MEADOW BROOKS
KY-3248
JC07
NOSCO ROAD
JC05
KY-37
PHILLIPS LANE
JC04
JC03
OLD US 127 LOOP NO 2 ROAD
NO NAME
JC08
CREAM RIDGE ROAD
White Oak Creek
JC09
US-127
Junction City
9 Sites (Exhibit 13)
HATCHER ROAD
KY-300
Knoblick Creek
Knoblick Creek
MST Sampling Site
Balls Branch
Clarks Run
Knob Lick Creek
White Oak Creek
3,600
0
3,600
Feet
N
Exhibit 1
Sampling Area of Clarks Run
and Hanging Fork Watersheds
Microbial Source Tracking Analysis
Dix River Watershed
Boyle County, Kentucky
Map Document (P:\2005\5167C-KDOW-DixSourceTrackMapping\GIS\EX1_Boyle_Co.mxd) 4/29/2008 -- 1:24:16 PM las

LINCOLN COUNTY

CASEY COUNTY

North Tributary of Hanging Fork 3 Sites (Exhibit 8)

Frog Branch 4 Sites (Exhibit 6)

Blue Lick Branch 4 Sites (Exhibit 5)

Baughman Branch 8 Sites (Exhibit 4)

Hanging Fork Below West Hustonville 6 Sites (Exhibit 9)

Hanging Fork Main Stem and Other Tributaries 9 Sites (Exhibits 10 & 11)

Peyton Creek 6 Sites (Exhibit 7)

McKinney Branch 5 Sites (Exhibit 3)

HF01 BL01 NO03 NO01 NO02 BL02 BL03 BL04 FR03 FR04 FR02 FR01 HF02 PE01 HF07 BA08 BA05 BA07 BA03 BA04 BA06 HF03 PE02 PE03 PE04 PE05 PE06 WH06 WH05 WH04 WH02 WH03 BA02 BA01 WH01 HF05 HF04 HF06 MC01 MC02 MC03 MC04 HF09 HF08 MC05

Harris Creek
Spears Creek
Baughman Creek
Frog Branch
Hanging Fork Creek
Peyton Creek
Martins Branch
Blue Lick Creek
McKinney Branch
NO NAME

KY-2141
KY-1194
KY-78
KY-906
KY-698
KY-1778
KY-198
US-127

MOORES LANE
HARRIS CREEK ROAD
VONLINGER ROAD
U DRIVE
NO NAME
OLD US 127 LOOP NO 2 ROAD
SAND KNOB ROAD
SPOONAMORE LANE
THORN LANE
BONEYVILLE ROAD
JEFFERIES ROAD
FROG BRANCH ROAD
CARTER SCHOOL ROAD
GORDON LICK ROAD
MAXEY VALLEY ROAD
BLACK PIKE
HOLTZCL
DALLAS DRIVE
DOUGLAS AVENUE
POWELL ROAD
BLUE LICK ROAD
NAYLOR ROAD
MARTINS TRAIL
OLD DANVILLE PIKE
STREET NO 1
GENEVA-N
CARPENTER ROAD
PECK HOLL
KIDS LANE

MST Sampling Site
Baughman Branch
Blue Lick Branch
Frog Branch
Hanging Fork Main Stem and Tributaries
McKinney Branch
North Tributary
Peyton Creek
West Hustonville

3,600 0 3,600 7,200 Feet

N

Exhibit 2
Sampling Area of Hanging Fork Watershed
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSource\Track\Mapping\GIS\EX2_Lincoln_Co.mxd) 4/29/2008 -- 1:30:50 PM las

E. coli and total coliform data will be reviewed and used to determine the "hotspots" or geographic areas with the highest concentrations of pathogen input. One primary and one secondary hotspot will be selected from each watershed division (with the exception of the main stem section from which 2 primary and 2 secondary sites will be selected) for subsequent analysis.

The Data Manager will deliver electronic data deliverables of all results in a COMPASS format to KDOW as they are completed and verified by the QA Manager.

1.4.5 Microbial Source Tracking of Host Sources

Once primary and secondary hotspots have been selected from each watershed, the Sampling and Laboratory Coordinator will schedule the collection of grab samples at each of the primary sites during a dry event, and at the primary and secondary sites during a wet event. Sample collection for these events will be conducted during the Primary Contact Recreation season.

The Sampling and Laboratory Coordinator will ship these samples to Source Molecular for laboratory analysis for the following parameters:

- Human Enterococcus ID
- Human Bacteroidetes ID
- Cow Enterococcus ID
- Cow Bacteroidetes ID

All samples that test positive for any of these parameters will be further analyzed by qPCR methodology to quantify the relative contribution of each host source to the total. While contributions from wildlife and domestic pets may also contribute to the contamination, the relative proportion of these sources may be ascertained by subtraction of the contributions from cattle and human from the total observed.

The Data Manager will deliver electronic data deliverables of all results in a COMPASS format to KDOW as they are completed and verified by the QA Manager.

1.4.6 Final Report

Third Rock's Project Administrator will deliver a final report to the KDOW entitled *Microbial Source Tracking in the Dix River Watershed*. Third Rock will use results from this study in conjunction with the 2005 monitoring analysis of Peyton Creek and Frog Branch and the 2006 through 2007 modeling study in the preparation of this report. The report will generally follow the outline listed below:

- Executive Summary
- Introduction
  - Background
    - Watershed Description
    - Problem Definition
    - Designated Use and Water Quality Standards
  - Goals and Objectives
  - Roles and Responsibilities
- Study Design
  - Site Location and Characterization
    - Land use
    - Geographic Location of Potential Sources
    - Human Wastewater Sources
  - Analytical Methods
  - Sample Collection
- Results and Data Analysis
  - Total Coliform and AC/TC Ratio Results
  - PCR and qPCR Results
  - Quality Control Results
- Assessment of Data and Pollutant Sources
  - Point Sources
  - Nonpoint sources
  - Failing Septic Systems
  - Livestock
  - Wildlife
  - Urban
  - Sewer
  - Etc.
- Conclusions and Recommendations for Remediation
- References
- Appendix A - Site Characterization Datasheets and Photo library
- Appendix B - Total Coliform Analytical Data
- Appendix C – PCR and qPCR Analytical Data

1.5 Quality Assurance Objectives

1.5.1 General Quality Objectives

The overall project data quality objective (DQO) is to identify and quantify the sources of pathogen pollution to facilitate effective remediation. Reaching this objective requires that data generated and used for modeling must be of sufficient quantity and quality to support:

- Identification of host sources in the area
- Geographical location of host sources

- Quantification of the relative contributions of host sources

The following items detail the quality objectives and performance criteria for the measurements associated with accomplishing these general objectives. The section on data quality objectives (DQOs) explains the criteria for determining whether data is of sufficient quality to support the goals of the project. The section on data quality indicators (DQIs) establishes the acceptance thresholds for the performance of each task.

As a result of this project, several outcomes may result. If sources are identified to a sufficient resolution and remediation is necessary, remediation plans will be incorporated into the Watershed Plan. If remediation is not necessary, then the problem has been successfully resolved. If sources are not determined to sufficient level resolution, additional studies may be conducted with a more focused geographical area, source identification, or temporal period.

1.5.2 Data Quality Objectives

1.5.2.1 GIS Data Set

Effective remediation of microbial pollution in the watershed requires elimination of the source(s) of pollution that pose the greatest risk for the spread of human disease. The high threat of the spread of disease from human fecal contamination is well documented, but the risk associated with animal feces is assumed to be lesser although few studies have confirmed this assumption (Field *et al.*, 2007). Knowing the importance of human wastewater contamination, information about the location of potential human wastewater sources and their likelihood of contamination is vital for effective remediation. Where visual observation can only account for straight pipes or other obvious human sources, leaking sewage pipes or outdated septic systems may not be readily detected without a GIS data set.

In general, the records compiled by the health department are secondary data, data previously collected for a different use. County health department records were collected to certify that new or remodeled residences or businesses meet environmental codes for onsite treatment. Files do not cover all facilities in the area, and often a lot number reference is used to mark the location since the dwelling or business is under construction at the time of inspection. For those facilities for which data is not available, reasons for the absence of data are to be recorded, if possible, in order to provide maximum insight into the status of these facilities.

In order for this secondary data to be useful in a GIS data set marking potential human wastewater sources, it must meet data quality objectives in four categories: 1) the type of facility, 2) the treatment type, 3) the age of the treatment system, and 4) location of the facility. Table 2, page 16, identifies the DQOs, criteria, and examples of satisfactory data for each of these four categories. A fifth category is also included, condition, for describing additional notes that may indicate an increased potential for watershed pollution.

TABLE 2 – GIS QUALITY OBJECTIVES

CATEGORY NAME	DQO	MINIMUM REQUIREMENT(S)	EXAMPLE(S)
Type of Facility	Provide an indication of the amount of human waste produced.	Type of dwelling or business. KPDES permitted facilities are not required.	single-family dwelling, multi-family dwelling (apartment, nursing home), business (small business, large business)
Type of Onsite Treatment	Describe the mechanics utilized to process the human wastewater onsite in order to evaluate the possibility for improper functioning.	Categorization as 1) sewer, 2) septic system, or 3) other. For septic systems, the notation of the alternative utilized should be noted if available. For treatments classified as "other", the reason for the classification should be noted.	1) Sewer 2) Conventional Septic Tank and Drainfields, Raised Bed and Septic Mound systems, Advanced Material Media Filtration systems, Aerobic Septic Systems, Wetlands and Constructed Wetlands, Disinfection Systems, Waterless and Low-Water and Greywater-Separation systems 3)Straight pipe, System unknown - constucted under Farmstead Exemption prior to 1983
Age of System	Indicates the need for maintenance, replacement, and potential for improper functioning.	Age in Years	1997, 10 years
Location	Geographical location to orient the system within the watershed.	Differentially corrected GPS observations in decimal degrees. Acceptable correction methods include NGS CORS/OPUS, local or community base station, or WAAS. Accurate to file data specifications. Provide metadata on how the location was found and its accuracy.	File indicates Lot # 3 in Fairbanks Neighborhood, platt cross-reference provides address is 2456 Fairbanks Road. GPS (Lat, Long) from 1 meter resolution FSA aerial imagery.
Condition	Additional notes in files of providing an indicator of the potential for contamination of the watershed.	Not required. Recommend recording if available	Violation March 15, 2006

1.5.2.2 Site Identification and Characterization

Site identification and characterization are the foundation upon which all subsequent source tracking measures are built. The number of locations and the spacing between them determines the amount of resolution possible for a given geographic area. The placement of site locations determines the efficiency of access as well as the representativeness of the sampling conducted in a given reach.

Project aerials were surveyed for a combination of variables including land use, site access, existing data implications, and potential source contributions to establish potential sites for characterization and analysis. However, visual observation of these sites is essential in their final establishment. Parameters to be considered in the selection of a site location include:

- Hydrological uniformity
- Proximity of other sites in the watershed and the differentiation of sources between them
- Representativeness of potential fecal sources in the reach
- Permission of land owners
- Depth of water throughout the sampling period
- Accessibility time
- Land use and area topography
- Potential for disturbance
- Repeatability of return visits

In establishing a site location, the Sampling Technician should apply professional judgment in considering the applicability of each of these parameters in the final site selection. Photographs, GPS waypoints, and field notations should provide sufficient documentation to support site selection decisions, aid in visualization of site conditions, and facilitate site location by other analysis parties.

The objective of the Microbial Source Tracking Site Characterization Datasheet (Appendix A) is to document visually observed fecal source contributors within the site area to sufficient detail to allow targeted remediation once contributions of the sources are assessed. While many fecal contributions are not observable due to the timing, location, or the method of the input, documentation of land use and subsequent MST analyses may aid in the detection of these sources. The detailed descriptions of sources in the area provide a basis for remediation and are essential in explaining the impacts in the watershed.

The objective of the Watershed Characterization Datasheet (Appendix B) is to provide a quantitative assessment of the environmental quality, a reference for measurement of remediation success, and reduce the uncertainty associated with non-quantitative assessment of stream conditions. According to Chapter 6 Section V of Kentucky Division of Water, 2002b, habitat assessment is subjective and therefore, the criterion for measurement of this parameter deserve further discussion.

The methodology recommends performing habitat assessment after the sampling so the investigator gains familiarity with the variation within the habitat. Although habitat assessment is scheduled for the beginning of the project, all Sampling Technicians have observed the watershed throughout an annual cycle and therefore have sufficient knowledge of variation within the streams to satisfy this criterion.

In order to decrease the subjectivity associated with the measurements, all Sampling Technicians will undergo training on habitat assessment by a qualified biologist. Ten percent of the sites (8 total) assessed will be evaluated by multiple Sampling Technicians in order to evaluate the precision of the measurements.

Physiochemical measurements are required to complete the Watershed Characterization Datasheet. The DQI associated with each physiochemical measurement is discussed in Section 1.5.3. However, a major source of uncertainty in the physiochemical measurements is the location and equilibration time of the multi-probe meter. Sampling Technicians shall ensure that the meter is placed in a representative area upstream of sampling disturbance. The meter shall be allowed to equilibrate until no significant change occurs in any parameter over a five second period. In this manner, the measurement will be representative of the stream under natural conditions and not biased due to disturbance or meter storage conditions.

1.5.2.3 Sampling Events

Sampling for total coliform, *E. coli*, and PCR methodologies will be conducted in a total of 4 events as described in Section 1.4. Collection is to occur during a "dry" and a "wet" event for each sample type. The timing and hydrologic stream conditions during these collection events are significant contributors to the project uncertainty.

Sampling is scheduled to occur during the Primary Contact Recreation Season, as described in the Kentucky Water Quality Standards. The objective of collection during this time period is to maximize the correlation of results to the human health risk associated with recreation water use. Additionally, livestock or other animal habits, movements, and feeding locations may change from warmer to colder months. Although multiple sample events throughout the Primary Contact Recreation Season would present a better picture of source contributions, project constraints do not allow for these events. In order to compensate for having fewer sampling events than optimal, Sampling Technicians must document the conditions observed in the watershed more extensively. Observations of cattle in or nearby the stream, deer tracks along the banks, or observed flow from a straight pipe, for example, at the time of the sampling event must be documented and/or photographed to ensure that these variables are sufficiently accounted for in the analysis. These observations, along with prior monitoring data over an extended period, should be sufficient to adjust the relative source contributions to an appropriate level.

Events are designated as either "dry" or "wet" in order to accomplish specific data objectives. A dry event is collected when medium to low flow is observed throughout the reach with some flow in the water. During dry event conditions, the objective is to capture the fecal contributions due to either direct deposition or leaching. Observations of the flow observed during collection should be noted. Stream conditions will meet a 10 to 14 day antecedent dry period to meet dry/low flow conditions.

A wet event is collected in association with the occurrence of a precipitation event. During a precipitation event, the objective is to capture the additional fecal contributions due to surface runoff and storm flow not present in a dry event. As such, the documentation of the precipitation at the time of or prior to sampling is significant. Stream conditions will meet a 3 to 7 day antecedent dry period, combined with a minimum of 0.2 inches of rain within a relatively short time period. Increased stream flow will be necessary before sample collection.

1.5.2.4 E.coli and Total Coliform Analysis

Since future remediation in the Dix River Watershed is ultimately aimed at reducing the health risk associated with pathogen loading, all variables associated with this risk are significant. Risk assessment can be summarized by the quantification of 3 variables: a source indicator, a loading indicator, and a fecal age indicator. *E.coli* sampling, in conjuction with the results of prior monitoring, will be utilized to indicate the loading of the watershed. The concentration of *E.coli* has been shown to more directly correlate with the pathogen risk than total coliform (USEPA, 1986) and therefore total coliform alone will not suffice as a loading indicator. The goal of the AC/TC ratio analysis associated with the total coliform is primarily to indicate the fecal age and secondarily indicate the source (PCR and qPCR primarily indicate the source). Together, these parameters can be used to access risk.

During field sampling for these parameters, variation may occur due to the sampling location within the stream, temporal variations, temperature, canopy cover, turbidity, chemical concentrations, and many other bacterial habitat-based variables. Within the laboratory, the growth of bacteria can vary due to temperature, incubation time, media, sterilization, and many other variables. Further, variability may be compounded when sub-sampling is required in order to dilute samples such that results are within the analysis range. Control of these variables is maximized through method specific quality control procedures.

Assessing acceptable overall quality for bacteriological parameters is difficult; Standard Methods does not provide criteria to evaluate acceptable recovery of a known sample for *E. coli* or total coliform. Therefore, an alternate method for establishing acceptable control criteria must be utilized in order to evaluate precision in field duplicate samples.

For this project, it is assumed that *E. coli* results could acceptably range from 50 percent to 200 percent of the reported result. This range is based on criteria established by annual proficiency testing studies in which statistical analysis is performed on known samples analyzed by multiple labs to establish acceptable recovery criteria.

1.5.2.5 AC/TC Ratio

Brion *et al.* (2000, 2002, and 2005) have established methods of evaluation of the age of fecal material based on the AC/TC ratio. Their results indicate that the higher the ratio of atypical colonies to typical colonies, the older the fecal input into the watershed is. An older fecal input presents less of a health risk due to the reduction in the number of potential pathogens.

Based on these studies, 5 categories have emerged indicating the source of the fecal inputs, as summarized in Table 3, on page 20. A ratio of 4 or below indicates fresh fecal matter from both humans and animals. Normally, ratios below 2 are characteristic of raw human sewage, active defecation by cattle can cause similarly low ratios. An AC/TC ratio between 4 and 10 indicates fecal matter most likely derived from indirect sources of agriculture, such as livestock. Indirect sources of urban runoff can range between 10 and 20; impounded urban runoff from 15 to 25. All ratios increase with time as the atypical coliforms proliferate and the total coliforms associated with fecal input die off. AC/TC ratios above 20 indicate aged fecal material from either human or agricultural sources.

TABLE 3 – RELATIONSHIP OF AC/TC RATIO AND SOURCE CONTRIBUTION

AC/TC RATIO	DESCRIPTION
< 2	Fresh, likely human source
2 - 4	Fresh, human or agriculture sources
4 -10	Moderate age, likely indirect agriculture
10- 20	Older, indirect urban
>20	Aged, human or agriculture sources

When measuring within a stream or watershed a decrease in the AC/TC ratio indicates a fresh input of fecal material and thus a nearby source. A low AC/TC result at a specific site may be caused by recent direct contributions from a number of sources, but in general the classifications above apply. Thus, if for example a AC/TC result of <2 is documented, then most likely there is a human sewage input in the area. Yet, because fecal inputs have temporal and geographic variability, documentation of fecal activities in the site area at the time of sampling is significant to meeting project objectives correlating AC/TC data to source inputs.

The resolution of this project allows for identification of sources throughout individual tributaries, but will not necessarily resolve the source locations along these tributaries. In sites where multiple source contributions along a tributary are not clarified by subsequent PCR source identification, more targeted and frequent sampling may be necessary to increase source resolution. Because a high AC/TC ratio would be the result of infrequent contributions, remediation or subsequent analysis should target areas of low AC/TC levels.

1.5.2.6 Hotspot Identification

The objective of the selection of hotspot sites for subsequent DNA analysis is to provide the maximum resolution to the largest problems within a watershed area. Primary hotspot sites will be chosen based on multiple variables including:

- Representativeness of overall watershed area
- Number of upstream sites
- *E. coli* concentration
- Number of potential sources
- Land use

Professional judgment will be utilized in the selection of the primary hotspot sites. It is expected that many primary sites will often be located at confluences or downstream locations within the watershed areas. The secondary sites will serve as compliments to the primary sites. As such, selection will be more influenced by factors such as concentration, potential sources, and land use.

1.5.2.7 Microbial Source Tracking of Host Sources

The goal of the PCR and qPCR analyses associated with this project is to identify the source contribution as human, bovine, or other and to quantify the relative contributions of each category. These analytical methods provide sensitivity to as little as one DNA marker per water sample.

The microbial groups used for fecal source tracking on this project will be the enterococci and the Bacteroidetes (Bacteroidales) associated with humans and cattle. The enterococci are currently used as water quality indicators and their presence in water has been correlated with epidemiological data to support their use as indicators of human health risk (Colford *et al.*, 2007; Wade *et al.*, 2003; Wade *et al.*, 2006). Host specific gene sequences within the enterococci have been used as targets for the source tracking methods (Scott *et al.*, 2005; Soule *et al.*, 2006). These sequences have been validated in numerous field studies conducted by Source Molecular's laboratory and the Human Enterococcus ID test has been validated in several published manuscripts and reports (Jenkins *et al.*, 2004; Liu *et al.*, 2006; McQuaig *et al.*, 2006; Scott *et al.*, 2005; USEPA, 2005). For the Bacteroidetes strains, several 16 S RNA DNA markers will be targeted that will differentiate between human and cattle fecal pollution. These gene targets have also been validated extensively in the literature (USEPA, 2005; McQuaig *et al.*, 2006; Shanks *et al.*, 2006a, 2006b).

The enterococci and Bacteroidetes markers will be targeted simultaneously for each fecal pollution source. By targeting these 2 microorganisms simultaneously, one can get a better appreciation of the type of fecal pollution present. Bacteroidetes are fecal anaerobes and lack the enzymes required for oxidative metabolism. Therefore, the presence of oxygen is toxic to these organisms and survival outside the host organism is reduced compared to microorganisms such as enterococci. As such, Bacteroidetes are useful complementary microorganisms for determining recent forms of fecal pollution. Furthermore, by using an additional microorganism, both negative and positive results can be validated with greater certitude (McQuaig *et al.*, 2006).

Although there is high confidence in the proposed test method, the small sample size may allow the presence of human or cattle inputs to be present in the watershed but not captured in the sample. Only with repeated sampling using multiple tests can one draw solid conclusions particularly with regard to negative results. Positive results can be judged with more leeway due to the high specificity of the methodology, but it is always prudent to confirm a positive result with another method, as proposed. Also, because of the high specificity of positive results, quantification of positive results exhibits a high degree of accuracy in contrast to the highly variable quantifications associated with library or culture based methodologies.

The proposed Host Specific PCR and qPCR methodologies prove to be useful indicators when judged against the criteria for a source indicator listed in the USEPA's *Microbial Source Tracking Guide Document* (USEPA, 2006). Below are detailed remarks related to the performance of these methodologies when compared against the ten method criteria listed in this guidance document.

1. Host-specific:

The Human Enterococcus ID, Human Bacteriodetes ID, Cow Enterococcus ID, Cow Bacteroidetes ID tests, and the quantifiable versions meet this criterion. As best as can be ascertained, all the DNA markers associated with the respective targeted microorganisms are highly host-specific. Furthermore, each marker can be quantified allowing proportional identification of the fecal pollution from each animal group. Evidence to this is provided in the references and in the attached reports.

2. Distribution of hosts:

All these markers/tests are found in all the members of the targeted population. The Human Enterococcus ID test is most useful as an indicator of the presence of domestic sewage and has been identified in 100 percent of sewage influent samples collected from throughout the continental US, Canada, and Europe. The marker has also recently been detected in the Hawaiian Islands, Alaska, and in New Zealand. It is not, however,

present in every human fecal sample. Therefore, it should not be used as an indicator of the presence of fecal pollution from either a single or several individuals. The other genetic markers (cow Enterococcus, human and cow Bacteroidetes) are more prevalent within the population. This observation is useful for determining not only sources but also types of fecal sources (*i.e.* septic tanks vs. domestic sewage).

3. Stability of pattern/marker:

The markers are highly stable and are not subject to mutation or methodological variations. Furthermore all of the gene targets are chromosomal and have not been conclusively shown to be present on exchangeable genetic vectors such as plasmids.

4. Temporal stability in host:

There is no temporal variability with these markers. The studies outlined in the supporting published reports and manuscripts have taken place over the past several years and over the period of months to years in multiple climate types over different seasons.

5. Geographic range / stability:

The markers are constant throughout a broad geographic range. The geographic range and stability of these markers has been well established for the continental US.

6. Representative sampling:

Relatively small sample sizes (100 to 200 milliliters of water) should be sufficient to identify the marker. Nonetheless, as with all environmental samples, only repeated sampling events can confirm the results.

7. Rate of decay:

The strains of microorganisms with their associated DNA markers proposed for this sample study have been shown to have a constant rate of decay. These microorganisms do not tend to survive very long in the environment since they are highly adaptive to their host organism. This is especially true for Bacteroidetes, but also true for the human and cattle associated enterococci.

It must be noted that other strains of enterococci have occasionally been shown to grow outside of the host organism (Whitman *et al.*); however; the enterococci strains to be used in this study have not exhibited this phenomenon. Because the targeted strains exhibit some degree of host specificity, it is believed that they have not retained the ability to live outside of their host for an extended period of time. The time of decay to extinction for the host specific fecal markers has been estimated to be up to 10 days for the Bacteroides spp. and up to 19 days for the enterococci.

8. Abundance in primary vs. secondary habitat:

As best can be ascertained, the distribution of the marker does not change after delivery to the water and this observation has been supported in bench survival studies.

9. Quantitative assessment:

The DNA markers proposed for this study can be quantified and the relative abundance of each type of fecal pollution targeted can be assessed from each water sample. Nonetheless, for proper interpretation of the quantifiable DNA markers, reference samples should be submitted from the surrounding geographic area of sampling. The reference samples (cow dung/slurry, human sewage, etc.) establish a baseline of how much of

the associated DNA markers are present in samples that are considered 100 percent representative of the targeted fecal pollution. Third Rock's methods of interpretation and quantification are not affected by dilution or bacterial loading from non-targeted hosts. The result is a quantifiable value (percentage) of bacteria in a water sample that are derived from a particular source.

10. Relevance to regulatory tools and health risk:

The enterococci markers can be easily correlated to the Kentucky water quality standards for pathogens. Enterococci are approved microorganisms by the EPA for determining permissible levels of fecal pollution and in establishing human health risks (Wade *et al.*, 2003; Wade *et al.*, 2006).

Although Bacteroidetes are not used for water quality standards, these microorganisms have shown to be good indicators of recent fecal pollution events. Furthermore, they have been shown to be excellent indicators of sources of fecal pollution. Although they should not be used on a stand-alone basis for microbial source tracking, they are excellent complementary indicators particularly to confirm recent fecal pollution and negative results.

To increase the certainty of the quantitative results, Source Molecular will analyze 2 reference samples for each of the DNA tests cited above. Two fresh cow slurry samples (composite samples preferred) and 2 raw sewage samples from nearby wastewater treatment facilities (composite samples preferred) will be submitted for analysis. The DNA marker levels from these reference samples will be compared to the DNA marker levels of the water samples. Comparing the DNA marker levels will allow a rough estimation of the percentage of fecal pollution from each respective source from each water sample. These samples will be submitted before the project starts to make sure that the molecular markers are being properly detected within the geographic region of the studied watershed.

In order to assess the uncertainty during this project, 2 unknown composite samples will be submitted to Source Molecular for quantitative analysis. These samples will be prepared similarly to the reference samples, but the laboratory will be blind to the source.

Due to the field variability associated with these testing, it should be noted that the representativeness of the sample will often determine the accuracy of the results to the larger site area. Only from repeated sampling using multiple tests can one draw solid conclusions particularly with regard to negative results. Positive results can be judged with more leeway due to the high specificity of the methodology and the secondary confirmatory methodology.

1.5.3 Data Quality Indicators

DQIs are qualitative or quantitative descriptors of data quality. The quality of field and analytical data is most often assessed in the following terms: precision, bias, accuracy, representativeness, comparability, completeness, and sensitivity. A review of these indicators follows.

For laboratory data, the laboratory performs the initial review of the results and compares them with the DQIs. Cause analysis and corrective actions are taken if necessary and deviations from the DQIs are noted with appropriate data qualifiers. The QA Manager performs a secondary review of the data to assess the conformance of the laboratory data in conjunction with field quality controls to the DQIs.

For field data, the Data Manager provides the initial review of data quality, and additional review is provided as the data is compiled and evaluated by the QA Manager. Table 4, page 25, contains method specific criteria for each DQI explained below.

1.5.3.1 Precision

Precision is the measure of agreement among repeated measurements of the same property under identical, or substantially similar conditions; calculated as either the range or as the standard deviation.

Precision uncertainties will be measured through the collection of field duplicate samples on 10 percent of the *E. coli* and total coliform samples. The laboratory additionally performs duplicate sample analysis with each analysis batch and is required to meet the requirements in Table 4, page 25.

The precision of RBP scores and general habitat assessment precision is controlled by the level of experience of the personnel conducting the assessment and with duplicate sampling at 10 percent (8) of the sites. All personnel involved in assessment have been trained to properly conduct these assessments.

Precision in PCR and qPCR methods in ensured by testing by multiple methodologies and through the use of triplicate analysis of DNA standards.

1.5.3.2 Bias

Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. Table 4, page 25, lists the biases of each method, if present, and the cause of these biases. For most methods, bias is incorporated into the uncertainty associated with the accuracy. For presence / absence PCR methods, false negatives can occur due to the small sample size and time period represented, but there is little to no generation of false positives.

1.5.3.3 Accuracy

Accuracy is a measure of the overall agreement of a measurement to a known value; it includes a combination of random error (precision) and systematic error (bias) components of both sampling and analytical operations. Accuracy will be determined through the use of quantitative samples of known value.

1.5.3.4 Representativeness

“Representativeness” is a qualitative term that expresses the degree to which a portion accurately and precisely represents the whole. Representativeness in the field is achieved by adherence to applicable KDOW, EPA, and/or published literature guidelines for sampling. Homogenization of a sample before analysis in the laboratory achieves representativeness. Samples are expected to be as representative as possible throughout the field and laboratory process.

TABLE 4 – METHODS, ANALYTES, AND DATA QUALITY INDICATORS FOR THE DIX RIVER WATERSHED

ANALYTE NAME	METHDOLOGY	UNITS	PRECISION DUP / FDUP (%RPD)	BIAS	ACCURACY LCS (±% UNCERTAINTY)	COMPARABILITY	COMPLETENESS	SENSITIVITY (IN SPECIFIED UNITS)
Turbidity	EPA 180.1	NTU	10	See Accuracy	20	High using same method	100%	differentiates to 0.01
pH	EPA 150.1	S.U.	5	See Accuracy	5	High using same method	100%	differentiates to 0.01
Dissolved Oxygen	EPA 360.1	mg/L	10	See Accuracy	20	High using same method	100%	differentiates to 0.01, LOQ = 1
Temperature	EPA 170.1	°F	5	See Accuracy	5	High using same method	100%	differentiates to 0.1
Conductivity	EPA 120.1	umhos/cm	10	See Accuracy	10	High using same method	100%	differentiates to 0.001, LOQ = 1
Rapid Habitat	KDOW, 2002b	#	10	Varies by experience	N/A	Regionally specific	100%	differentiates to 1
Total Coliform, AC/TC	SM 9222B mod. as in Brion 2005	#/100mLs	20 / 50-200%*	See Accuracy	50-200%	High using same method	100%	1 X Dilution Factor
E. coli	EPA 1603	#/100mLs	20 / 50-200%*	See Accuracy	50-200%	High using same method	100%	1 X Dilution Factor
Human Enterococcus ID	Source Molecular Internal SOP	Detection	N/A	False negatives can occur due the small sample size and time period.	Presence/Absence	High with presence / absense enterrococcus tests	100%	1 DNA marker / sample
Human Enterococcus Quantification	Source Molecular Internal SOP	CFU /100ml (Equivalent)	10	Highly specific	10	High with enterrococcus quantification tests; assumes reference sample from watershed	All positives	2000 copy / L
Cow Enterococcus ID	Source Molecular Internal SOP	Detection	N/A	False negatives can occur due the small sample size and time period.	Presence/Absence	High with presence / absense enterrococcus tests	100%	1 DNA marker / sample
Cow Enterococcus Quantification	Source Molecular Internal SOP	CFU /100ml (Equivalent)	10	Highly specific	10	High with enterrococcus quantification tests; assumes reference sample from watershed	All positives	1500 copy / L
Cow Bacteroidetes ID	Source Molecular Internal SOP	Detection	N/A	False negatives can occur due the small sample size and time period.	Presence/Absence	High with other presence / absense bacteroidetes tests, but may not detect inputs as old as enterococcus test.	100%	1 DNA marker / sample
Cow Bacteroidetes Quantification	Source Molecular Internal SOP	CFU /100ml (Equivalent)	10	Highly specific	10	High with bacteroidetes quantification tests; assumes reference sample from watershed, will not detect inputs as old as enterococcus	All positives	2000 copy / L
Human Bacteroidetes ID	Source Molecular Internal SOP	Detection	N/A	False negatives can occur due the small sample size and time period.	Presence/Absence	High with other presence / absense bacteroidetes tests, but may not detect inputs as old as enterococcus test.	100%	1 DNA marker / sample
Human Bacteroidetes Quantification	Source Molecular Internal SOP	CFU /100ml (Equivalent)	10	Highly specific	10	High with bacteroidetes quantification tests; assumes reference sample from watershed, will not detect inputs as old as enterococcus	All positives	500 copy / L
Definitions: RPD = Relative Percent Difference LCS = Laboratory Control Sample LOQ = Limit of Quantitation	DUP = Duplicate FDUP = Field Duplicate		*The 50-200% of each the sample must overlap the range for the FDUP.					

1.5.3.5 Comparability

Comparability is a qualitative term that expresses the measure of confidence that one data set can be compared to another and can be combined for decisions to be made. Comparability of *E.coli* and total coliform results will be ensured through strict adherence to KDOW and EPA sampling and laboratory methods. PCR and qPCR methods are comparable to similar methods utilizing the same host organism. qPCR methods also are comparable from region to region as percentages, but quantitatively are specific to the region from which the reference sample was collected. Comparability of physio-chemical results will be ensured through regular probe calibration. Comparability of habitat data will be ensured through strict adherence to sampling protocols developed by the KDOW for in-stream habitat.

1.5.3.6 Completeness

Completeness is a measure of the amount of valid data to be obtained from a measurement system. It is expected that planned sampling will be 100 percent completed unless stream sites dry throughout the sampling period. However, if 100 percent of sites cannot be sampled, the number of sites sampled must be sufficient to characterize the watershed.

For the GIS data set, the goal for completeness is to locate all facilities within Lincoln and Boyle counties for which there is data.

1.5.3.7 Sensitivity

Sensitivity is the capability of a method or instrument to discriminate between measurement responses representing different levels of variable interest. Sensitivity for this project is achieved by adherence to the limits of quantitation listed in Table 4, page 25. Limits of quantitation are determined by a calculation based upon the detection limit for analytical methods and instrumentation.

1.6 Documentation and Records

1.6.1 General

In order to provide quality consulting to the KDOW, traceability and maintenance of documentation and records are essential. All records relating in any manner whatsoever to the project, or any designated portion thereof which are in the possession of Third Rock shall be made available, upon request of the KDOW. Additionally, these records shall be available to any applicable regulatory authority and such authorities may review, inspect and copy these records. These records shall be retained for at least 3 years after the project is approved and closed by the EPA.

This section will examine the documentation that will be utilized or produced during this project and the responsibilities for the maintenance and delivery of these documents or records. In general, project documents may be divided into categories: operational, deliverable, and internal. Table 5, page 27, lists the project documents and records that fall under each of these categories. Further information on each of these documents and records is provided below.

TABLE 5 – PROJECT DOCUMENTS AND RECORDS

OPERATIONAL	DELIVERABLE	INTERNAL
QAPP	GIS Data set	Instrument Calibration Logs
Source Molecular's Quality Policy Manual	Chain-of-Custody Forms	Instrument Maintenance Logs
Standard Operating Procedures	Watershed Characterization Datasheet	Laboratory Datasheets
Field and Laboratory Datasheets	Microbial Source Tracking: Site Characterization Datasheet	Sample Labels
	Laboratory Results	Billing Receipts
	Meeting Minutes	Project Email Documentation
	COMPASS Electronic Data Deliverable	
	Final Report	

1.6.2 Operational Documents and Records

1.6.2.1 QAPP Management and Distribution

Key to these goals is the distribution of the most recent version of this QAPP to all parties listed on the distribution list once the QAPP has been reviewed and approved. The QA Manager is responsible for ensuring that all applicable parties perform documented review of the QAPP. If, because of deviations in the QAPP, revisions are required, the QA manager shall ensure that all parties review the revised version. The current revision and the date of the revision shall be documented in the upper right corner of the QAPP pages. The QAPP shall be redistributed after all parties have reviewed the document.

1.6.2.2 Source Molecular Document Control

Source Molecular maintains a current Quality Policy Manual document all aspects of their quality system including control of Standard Operating Procedures (SOPs) and datasheets. According to the Policy Manual, "Documents issued as part of the quality system are reviewed and approved by authorized personnel. A master list identifying the current revision and distribution of documents in the quality system is used to ensure invalid and obsolete documents are not used." Quality system documents are uniquely identified by the date of the last revision, issuing authority, and the total number of pages or a mark indicating the end of the document. The current Quality Policy Manual is available in Appendix C.

1.6.2.3 Third Rock Document Control

Third Rock maintains control of all SOPs, datasheets, and quality documentation through a limited access disk drive. All current datasheets and SOPs are accessible via the internal Anet and are printed out as needed for use. These documents are uniquely identified by their title and revision date.

Revision of documentation is conducted as needed to ensure accuracy. The document database manager provides a copy of the original file to the technical editor for revision. Before finalization, any revisions to the document must undergo a technical peer review and an administrative review before it is posted for use.

1.6.3 Deliverable Documents and Records

Deliverable documents include all documents that will be provided to the KDOW during the course of this project.

1.6.3.1 GIS Data Set

The GIS data set will be produced in a Microsoft Excel Format under the direction of the GIS Manager. At minimum, this data set will have columns for each of the 5 categories: type of facility, type of onsite treatment, age of system, location, and condition. If necessary, additional columns may be added to aid in organization. The GIS data set will be stored in the project files which are electronically backed-up daily.

The Project Administrator will deliver the GIS data set to the KDOW subsequent to verification and analysis by the QA Manager.

1.6.3.2 Field and Laboratory Data Report Package

A Data report package will consists of field data, chain-of-custody forms, and analytical laboratory reports. Specifically the final package will include copies of the following:

- Field observations recorded in the Sampling Technicians' Field Notebook (if necessary)
- Watershed Characterization Datasheet (Appendix B)
- Microbial Source Tracking: Site Characterization Datasheet (Appendix A)
- GPS Positioning and Photographs
- Completed Chain-of-Custody Forms (Appendix D)
- Analytical Laboratory Reports (Appendix E)
- Final Report

Data reporting packages will contain a consistent format and will be compiled initially during the quarterly meetings with KDOW and ultimately within the final report. Electronic copies of all hardcopy data listed above will be stored in Third Rock's project files. Electronic data will be presented in Adobe Acrobat, Microsoft Excel, and/or Access (COMPASS format) depending on the data type.

The original copies of all field notes, field data sheets, lab sheets, chain-of-custody forms, and lab reports will be maintained and stored at Third Rock for the required document retention period for the grant. The Sampling and Laboratory Coordinator will oversee this document control. At the end of the required period, the documents will be archived in Third Rock's warehouse. Copies of all electronic data will be archived in specified Third Rock computer files.

Third Rock will also deliver a final report using the format outlined in Section 1.4.6 Final Report.

1.6.3.3 Meeting Minutes

Briefing meetings are scheduled to occur quarterly or as needed. The Project Administrator will delegate transcription of the minutes from these meetings for submission to KDOW. All meeting minutes will be stored in Third Rock's internal project files.

1.6.3.4 COMPASS Electronic Data Deliverable

Third Rock will also deliver analytical data in a COMPASS format for all sampled stations. The guidance provided in the *Technical Support Document For Compass Electronic Data Delivery For Contracting Organizations Version: 8-May-08* and the tables provided in the Access database file entitled *Compass Field + Lab Data Template for Outside Organizations* will be followed by the Data Manager.

1.6.4 Internal Documents and Records

Internal documents is a description of all recorded data that is not submitted in a deliverable or is not operational in its scope, but is necessary in supporting the deliverable information. Internal documents include instrument calibration and maintenance logs, completed laboratory datasheets, sample labels, billing receipts, and project related emails.

All internal documents and records will be maintained and stored at Third Rock for the required document retention period for the grant. At the end of the required period, the documents will be archived in Third Rock's warehouse. Copies of all electronic data will be archived in specified Third Rock computer files. Source Molecular will also maintain all records associated with the analytical results including laboratory notebooks, bench sheets, instrument calibration and sequence logs, preparation logs, maintenance logs, etc. for the retention period of the grant.

2 Data Generation and Acquisition

2.1 Sampling Process Design

The sampling process design to be use in this investigation is a multistage, judgmental design. This sampling design is necessary because the project incorporates a large geographical area in a short time schedule on a limited budget. The area of investigation covers 27,800 acres in the Hanging Fork Watershed and 2,200 acres in the Clarks Run Watershed with a time schedule of one year or less to completion. Use of a statistical sampling design would require a larger number of sampling sites at a larger frequency to make conclusions as to the source identifications and relative contributions in the watershed.

In diagnosing the sources of microbial pollution, the cost of methodologies in comparison to the information value of the data they produce is a key factor in determining which methods are utilized and to what degree in the sampling process. In order to provide the most information for the least cost, multiple stages of testing using progressively more discriminating methodologies are recommended. In this manner, inexpensive testing methodologies can be utilized to rule out suspected source contributions that are not present and more discriminating methodologies can

quantify and discriminate between sources that are present. Using this principle, Third Rock plans to diagnose the source contributions to the Dix River Watershed with a four level approach as outlined below. Each of these stages of analysis is a logical stepping stone to the next stage, and ensures that the best cost to information ratio is produced.

1. Site Identification and Characterization
2. *E. coli* and Total Coliform Analysis for Hotspot Identification
3. Presence/ Absence PCR for Cattle and Humans by:
   - 3.1 Human Enterococcus ID
   - 3.2 Human Bacteroidetes ID
   - 3.3 Cow Enterococcus ID
   - 3.4 Cow Bacteroidetes ID
4. qPCR for Positive Cattle and Human Samples

GIS Data Set:

The development of the GIS dataset for human wastewater sources is separate task that compliments the multi-stage process. GIS data set will sample the Boyle and Lincoln County areas. The majority of the watershed divisions under investigation are located in Boyle or Lincoln Counties, with only a small portion of the source waters for Baughman Creek and the Hanging Fork below West Hustonville located in Casey County. The location of these human sources within the watershed will aid locating specific site locations in order to best characterize these sources, as applicable.

Site Identification and Characterization:

Site identification and characterization, as the first stage, will provide the initial visual screening of sources in the area. Seventy-four sites have been proposed, located within 11 watershed divisions, in order to facilitate identification and analysis. Table 6 identifies the number of sites located within each of the watershed division and the station code unique to each division. Each site is uniquely identifiable by the station code and a unique 2-digit number. Each of these watershed divisions is described further on pages 30 to 31.

TABLE 6 – STATION CODES AND SITE NUMBERS BY WATERSHED DIVISION

WATERSHED DIVISIONS	NUMBER OF SITES	STATION CODE	EXHIBIT NUMBER
Within Hanging Fork			
McKinney Branch	5	MC	Exhibit 3
Baughman Branch	8	BA	Exhibit 4
Blue Lick Branch	4	BL	Exhibit 5
Frog Branch	4	FR	Exhibit 6
Peyton Branch	6	PE	Exhibit 7
Northern unnamed tributary of Hanging Fork	3	NO	Exhibit 8
Hanging Fork below West Hustonville	6	WH	Exhibit 9
Hanging Fork main stem and other tributaries	9	HF	Exhibits 10-11
Within Clarks Run			
Balls Branch West	7	BB	Exhibit 12
Junction City	9	JC	Exhibit 13
Clarks Run	13	CR	Exhibits 14-15
Total	74		

1. McKinney Branch

The McKinney Branch Watershed Division describes the watershed drained by the McKinney Branch of the Hanging Fork in Lincoln County. The confluence of McKinney Branch and Hanging Fork is located just west of the intersection of KY-78 and McKinney-Chicken Bristle Road. The watershed division begins at the historic McKinney site location used during the TMDL watershed monitoring. Five sites are located in the McKinney Branch area accessible by McKinney-Chicken Bristle Road and Short Pike Road. Land use is largely agricultural with some residential use in the southeast at the town of McKinney.

2. Baughman Branch

The Baughman Branch Watershed Division is located along the western edge of the Hanging Fork watershed in Lincoln and Casey counties and is drained by Baughman and Spears Creek and their tributaries. The confluence of Baughman Creek and Hanging Fork occurs just southeast of the intersection of US-127 and KY-78. The watershed area contains 8 sites accessible by KY-78, Holtzclaw Lane, Black Pike, and Carter School Road. In order to capture the sources attributed to multiple tributaries within the watershed, some site locations are remotely located and are accessible though access from private drives or by walking along the creek banks. Land use in the watershed is primarily agricultural with some forested area in the northwest and some residential use in the east.

3. Blue Lick Branch

The Blue Lick Branch Watershed Division is located in the eastern part of the Hanging Fork Watershed in Lincoln County and is drained by Blue Lick Creek and its tributaries. The confluence of Blue Lick Creek and Hanging Fork occurs just north of the intersection of Blue Lick Creek and KY-1194. The 4 sites in the watershed division are accessible by KY-1194, Thornhill Lane, US-78, Boneyville Road, and Blue Lick Road. Land use is primarily agricultural with some forested areas in the northwest and southeast corners of the watershed.

4. Frog Branch

The Frog Branch Watershed Division is located centrally within the Hanging Fork Watershed in Lincoln County, and is drained by Frog Branch and its tributaries. The confluence of Frog Branch and Hanging Fork occurs just southwest of the intersection of S. Elliot Road and Frog Branch Road (KY-198). The 4 sites within the watershed can be accessed by S. Elliot Road, Frog Branch Road (KY-198), Peyton Well Road, and a private drive located to the south of Dotson Dr. (just off of US-127). Land use is primarily agricultural with some residential to the west near US-127.

5. Peyton Branch

The Peyton Branch Watershed Division is located in the south east of the Hanging Fork Watershed and is drained by Peyton Creek, Martins Branch, and their tributaries. Although the confluence of Hanging Fork and Peyton Creek occurs more northward, this watershed division will only involve the watershed area located downstream of the first site, located along KY-198. All watershed area downstream of this site is included in the Hanging Fork main stem watershed division. Frog Branch Road (KY-198), Blue Lick Road, and KY-78 provide access to the 6 sites in the area. Land use in the area is agricultural.

6. Northern Unnamed Tributary of Hanging Fork

Three sites are located in this watershed area found along the first tributary to Hanging Fork found south of the historic McCormick church site used in the TMDL watershed monitoring. Site locations are accessible by Peyton Well Road and KY-1194. Land use is primarily agricultural in this area.

7. Hanging Fork Below West Hustonville

The Hanging Fork Watershed area upstream of the historic West Hustonville site for the TMDL Watershed Study has been divided for investigation in this study. The West Hustonville site is located just west of the confluence of Hanging Fork and Baughman Creek at the intersection of US-127 and KY-78. The 6 sites within this watershed area are accessible from KY-78, Black Pike, Maxeys Valley Road, and KY-906. The watershed area spans both Casey and Lincoln Counties and the land use is primarily agricultural with some forested use to the west.

8. Hanging Fork Main Stem and Other Tributaries

The Hanging Fork Watershed area located upstream of the historic McCormick Church site and not classified into another watershed division will be located within this largest division of the watershed. All other watershed areas except the Blue Lick and Balls Branch West division are tributaries into this area. 9 sites are located within this watershed area in Lincoln County. These sites may be accessed primarily by KY-78, but also by KY-518, Hensley Lane, McKinney-Chicken Bristle Road, and Country Lane.

9. Balls Branch West

The Balls Branch West Watershed Division is located in the Clarks Run Watershed, south of Danville. Balls Branch, and its tributaries upstream of the historic Balls Branch West site, drain the watershed area located along the Boyle–Lincoln County line. The 7 sites in the area are accessible by Gose Pike, US-127, Hustonville Road, and McBee Drive. Land use in the area is divided between residential and agricultural.

10. Junction City

The Junction City Watershed Division is located in the Clarks Run Watershed, near Junction City. Knoblick Creek, White Oak, and their tributaries drain the watershed area. Nine sites are located in this area, however the historic White Oak and Junction City sites are not included in these locations. Sites are accessible via US-127, KY-300, Sycamore Street, KY-37, and Nosco Road.

11. Clarks Run

Located in and around Danville, the 13 Clarks Run sites are accessible by main roadways with main roadways including KY-52, US-127, KY-34, and US-127 Bypass. This division of the Clarks Run watershed is upstream of the historic Clarks Run Division of Water sampling site and includes six historic sites in the area.

The proposed location of each site is shown on Exhibits 3 through 15, on pages 33 through 45.

Visual observation of these sites is essential in their final establishment. Considerations to be documented in establishing the final site locations include:

- Hydrological uniformity
- Proximity of other sites in the watershed and the differentiation of sources between them
- Representativeness of potential fecal sources in the reach
- Permission of land owners
- Depth of water throughout the sampling period
- Accessibility time
- Land use and area topography
- Potential for disturbance
- Repeatability of return visits

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex3_McKinney_Br_Sites.mxd) 4/29/2008 -- 2:14:05 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

N

HF03
HF04
HF05
HF06
MC01
MC02
MC03
MC04
MC05

Hanging Fork Creek
McKinney Branch

KY-78
KY-198
KY-1778
STREET NO 2
STREET NO 4
BONEYARD HOLLOW ROAD
STREET NO 8
STREET NO 3
STREET NO 1
STREET NO 7
RAILROAD STREET
STREET NO. 9
GERMAN MCKINNEY ROAD

MST Sampling Site
Hanging Fork Main Stem and Tributaries
McKinney Branch

1,250 0 1,250 2,500
Feet

Exhibit 3
McKinney Branch Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

N

BA08
BA05
BA07
BA06
BA04
BA03
BA02
BA01
HF07
WH06
WH05
WH04
WH02
WH03
WH01

CASEY COUNTY
LINCOLN COUNTY

BUTCHERTOWN ROAD
BLAIR MILL ROAD
CARTER SCHOOL ROAD
GORDON LICK ROAD
MAXEY VALLEY ROAD
CARTER SCHOOL ROAD
BLACK PIKE
Baughman Creek
Spears Creek
Hanging Fork Creek
KY-906
KY-78
SPEARES LANE
NOVA LANE
THOMAS LANE
GREG DRIVE
OLD US 127 (OOP NO 2 ROAD)
JEFFERIES ROAD
HOLT-CLAN LANE
FAIRLANE AVENUE
SUNSET BOULEVARD
KY-2141
US-127
DALLAS DRIVE
CHILDRESS STREET
CARTER AVENUE
SOUTH 1ST STREET
SOUTH 2ND STREET
SOUTH 3RD STREET
HANCOCK STREET
Hanging Fork Creek

MST Sampling Site
Baughman Branch
Frog Branch
Hanging Fork Main Stem and Tributaries
West Hustonville

1,300 0 1,300 2,600
Feet

Exhibit 4
Baughman Branch Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex4_Baughman_Br_Sites.mxd) 4/29/2008 -- 2:20:07 PM las

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex5_BlueLick_Br_Sites.mxd) 7/11/2007 -- 2:18:54 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

N

BL01

KY-1194

KY-78

BL02

NO NAME

THORNHILL LANE

BL03

BL04

SPOONAMORE LANE

BONEYVILLE ROAD

Blue Lick Creek

BLUE LICK ROAD

WEST SKYLINE DRIVE

NO NAME

MST Sampling Site

Blue Lick Branch

Hanging Fork Main Stem and Tributaries

Peyton Creek

1,250 0 1,250 2,500

Feet

Exhibit 5
Blue Lick Branch Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

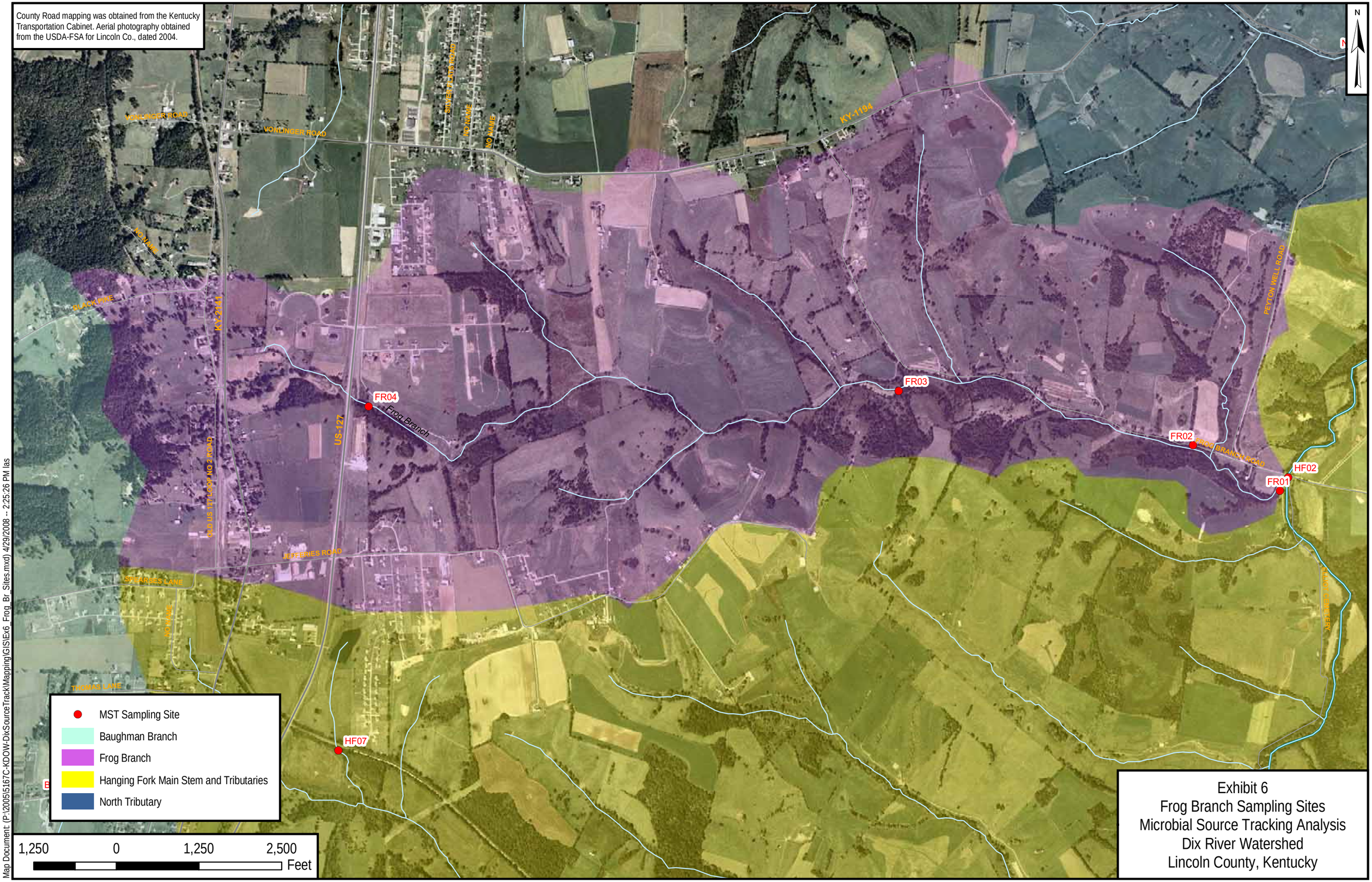


Exhibit 6
Frog Branch Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

Map Document: (P:\2005\5167C-KDOW-Dix\SourceTrack\Mapping\GIS\Ex6_Frog_Br_Sites.mxd) 4/29/2008 -- 2:25:26 PM las

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex7_Peyton_Br_Sites.mxd) 4/29/2008 -- 2:31:55 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

N

PE01
PE02
PE03
PE04
PE05
PE06

TRACE BRANCH ROAD
KY-78
NO NAME
BLUE LICK ROAD
NO NAME
Martins Branch
MARTINS TRAIL
NAYLOR ROAD
Peyton Creek
KY-196
PECK HOLLOW ROAD

MST Sampling Site
Blue Lick Branch
Hanging Fork Main Stem and Tributaries
McKinney Branch
Peyton Creek

1,250 0 1,250 2,500
Feet

Exhibit 7
Peyton Branch Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

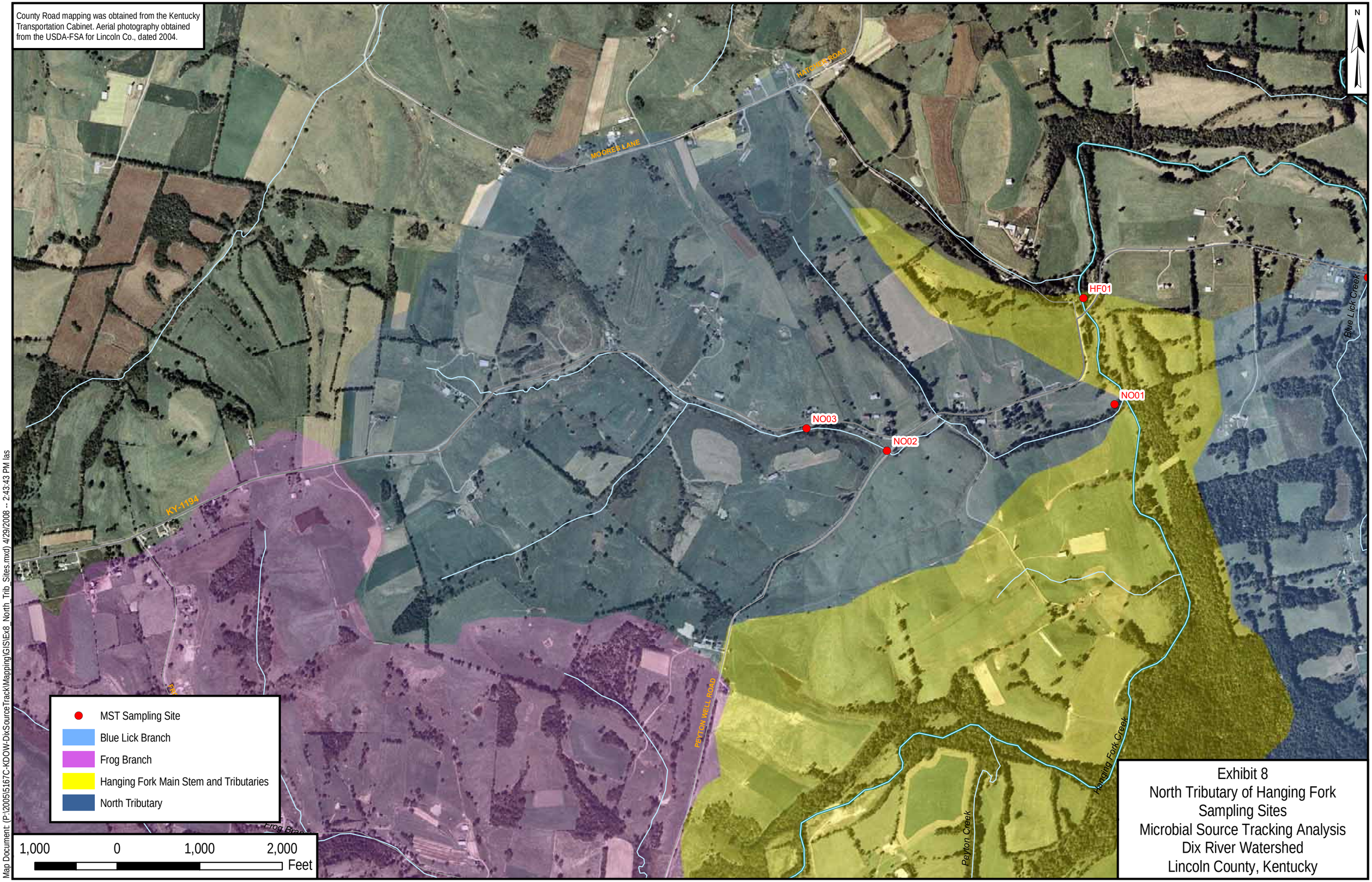


Exhibit 8
North Tributary of Hanging Fork
Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex8_North_Trib_Sites.mxd) 4/29/2008 -- 2:43:43 PM las

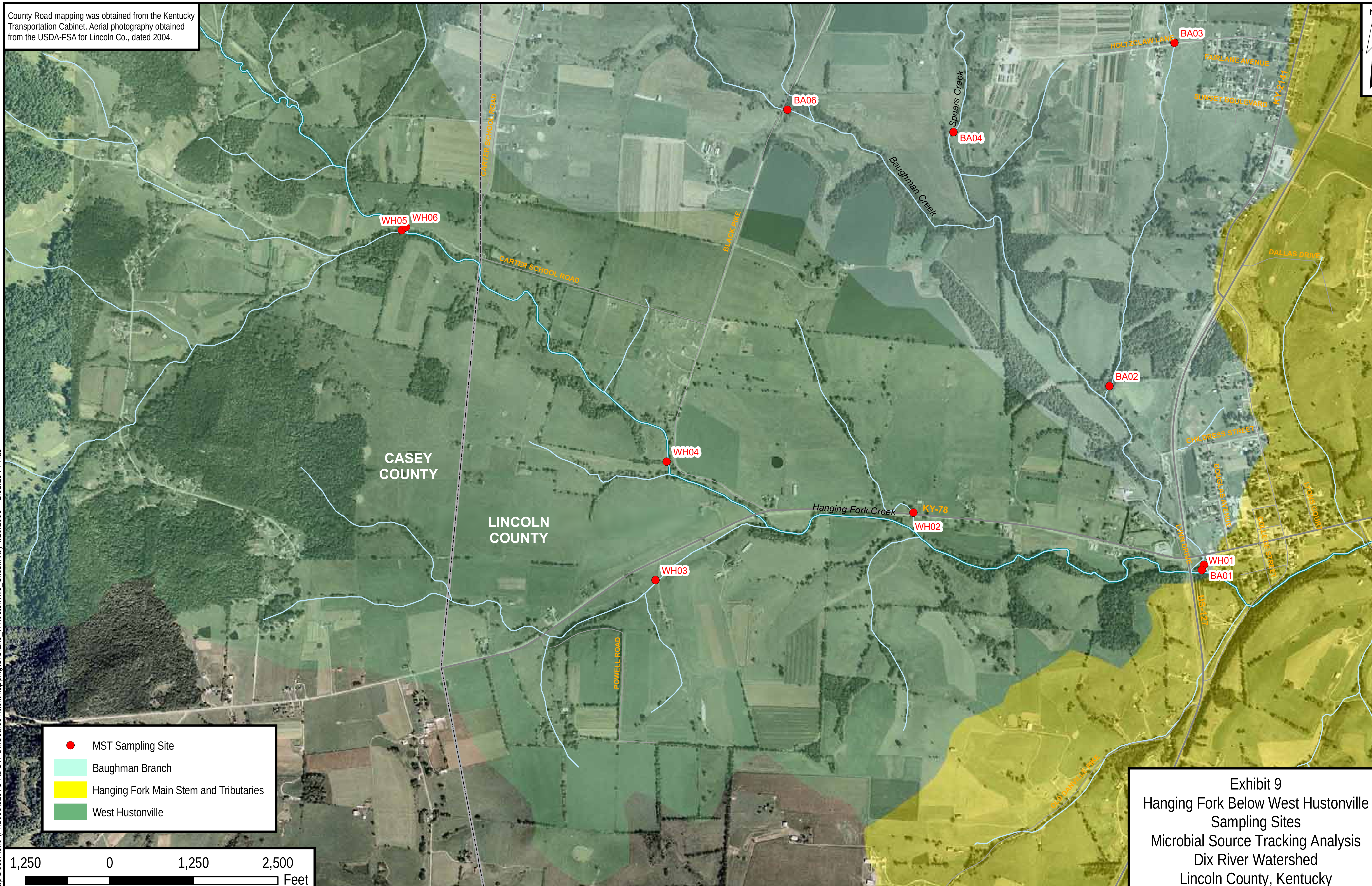


Exhibit 9
Hanging Fork Below West Hustonville
Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrackMapping\GIS\Ex9_WHustonville_Sites.mxd) 4/29/2008 -- 2:51:13 PM las

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex10_Hanging_Fk1_Sites.mxd) 4/29/2008 -- 2:55:23 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

N

MOORES LANE

HF01

NO01

NO03

NO02

KY-1194

Hanging Fork Creek

PEYTON WELL ROAD

Peyton Creek

Frog Branch

FR02

HF02

FR01

FROG BRANCH ROAD

ELLIOT CEMETERY

PE01

PE02

PE03

HF03

KY-78

HF05

HF04

MC01

1,250 0 1,250 2,500 Feet

MST Sampling Site
Blue Lick Branch
Frog Branch
Hanging Fork Main Stem and Tributaries
North Tributary
Peyton Creek

Exhibit 10
Hanging Fork Sampling Sites 1-5
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Lincoln Co., dated 2004.

N

THOMAS LANE
GREG DRIVE
HOLTZCLAW LANE
FAIRLANE AVENUE
SUNSET BOULEVARD
KY 2741
US-127
DALLAS DRIVE
CHILDRESS STREET
DOUGLAS AVENUE
COLLEGE STREET
DONOUGH
HANCOCK STREET
SPRUCE STREET
Baughman Creek
KY 78
Hanging Fork Creek
McKinney Branch

HF07
BA03
BA02
WH01
BA01
HF09
HF08
HF06
HF05
HF04
HF03
MC01
MC02
MC03
MC04

MST Sampling Site
Baughman Branch
Hanging Fork Main Stem and Tributaries
McKinney Branch
West Hustonville

1,250 0 1,250 2,500 Feet

Exhibit 11
Hanging Fork Sampling Sites 3-9
Microbial Source Tracking Analysis
Dix River Watershed
Lincoln County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex11_Hanging_Fk2_Sites.mxd) 4/29/2008 -- 3:00:46 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Boyle Co., dated 2004.

N

ARNOLD ROAD
POPPLEWELL ROAD
BONTA LANE
CRESTVIEW DRIVE
WOODSTOCK DRIVE
OLD DANVILLE ROAD
PARK HILLS DRIVE
Balls Branch
Cedar Fork
BB01
BB02
BB03
BB04
BB05
BB06
BB07
BOYLE COUNTY
LINCOLN COUNTY
CHANDLER DRIVE
US-127
EAST FRANCK DRIVE
WEST M.A. AVENUE
EAST M.A. AVENUE
HOWARD PLACE
WEST MEIGS LANE
RAILROAD STREET
CRAB ORCHARD STREET
CRAB ORCHARD STREET
PEMBERTON LANE
EAST MEIGS LANE
KY-37
KY-1500
WEST GRUBER LANE
EAST GRUBER LANE
GAS STREET
LAUSMAN AVENUE
PREARIN AVENUE
KY-1273
TUCKER LANE
SIMPSON LANE
JOHNSON LANE
ALBANY CIRCLE
DENKAN DRIVE
ALBANY DRIVE
KENTUCKY AVENUE
Clarks Run
Knob Lick Creek

MST Sampling Site
Balls Branch
Clarks Run
Knob Lick Creek
White Oak Creek

1,250 0 1,250 2,500 Feet

Exhibit 12
Balls Branch West Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Boyle County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrackMapping\GIS\Ex12_Balls_Br_West_Sites.mxd) 4/29/2008 -- 3:14:51 PM las

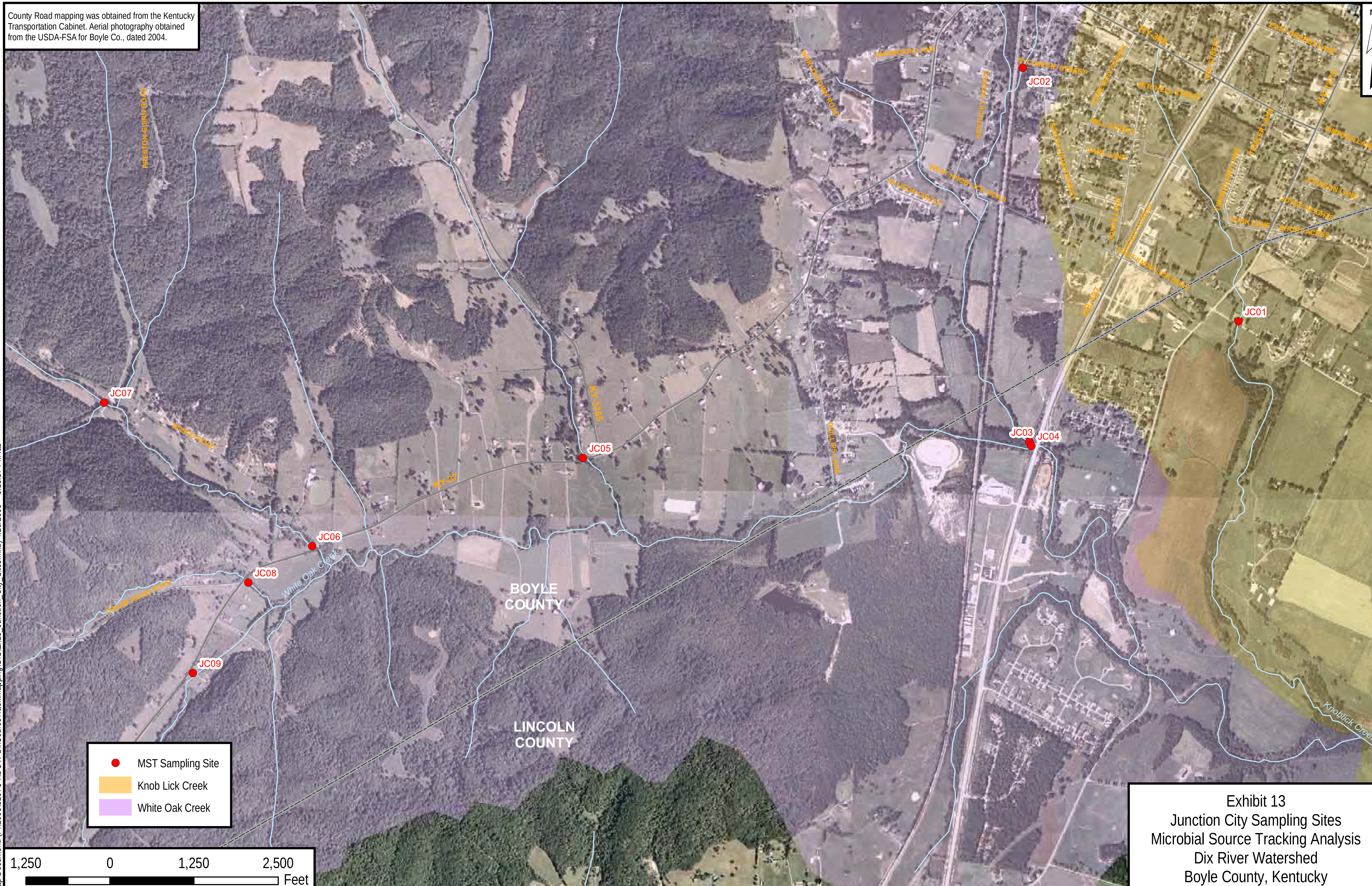


Exhibit 13
Junction City Sampling Sites
Microbial Source Tracking Analysis
Dix River Watershed
Boyle County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrackMapping\GIS\Ex13_Junction_City_Sites.mxd) 4/29/2008 -- 3:18:37 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Boyle Co., dated 2004.

N

CR01
CR02
CR03
CR04
CR05
CR06
CR07
CR08
CR09
CR10

MST Sampling Site
Balls Branch
Clarks Run

1,250 0 1,250 2,500
Feet

Exhibit 14
Clarks Run Sampling Sites 1-10
Microbial Source Tracking Analysis
Dix River Watershed
Boyle County, Kentucky

Map Document (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex14_Clarks_Run_Sites.mxd) 4/29/2008 -- 3:23:29 PM las

County Road mapping was obtained from the Kentucky Transportation Cabinet. Aerial photography obtained from the USDA-FSA for Boyle Co., dated 2004.

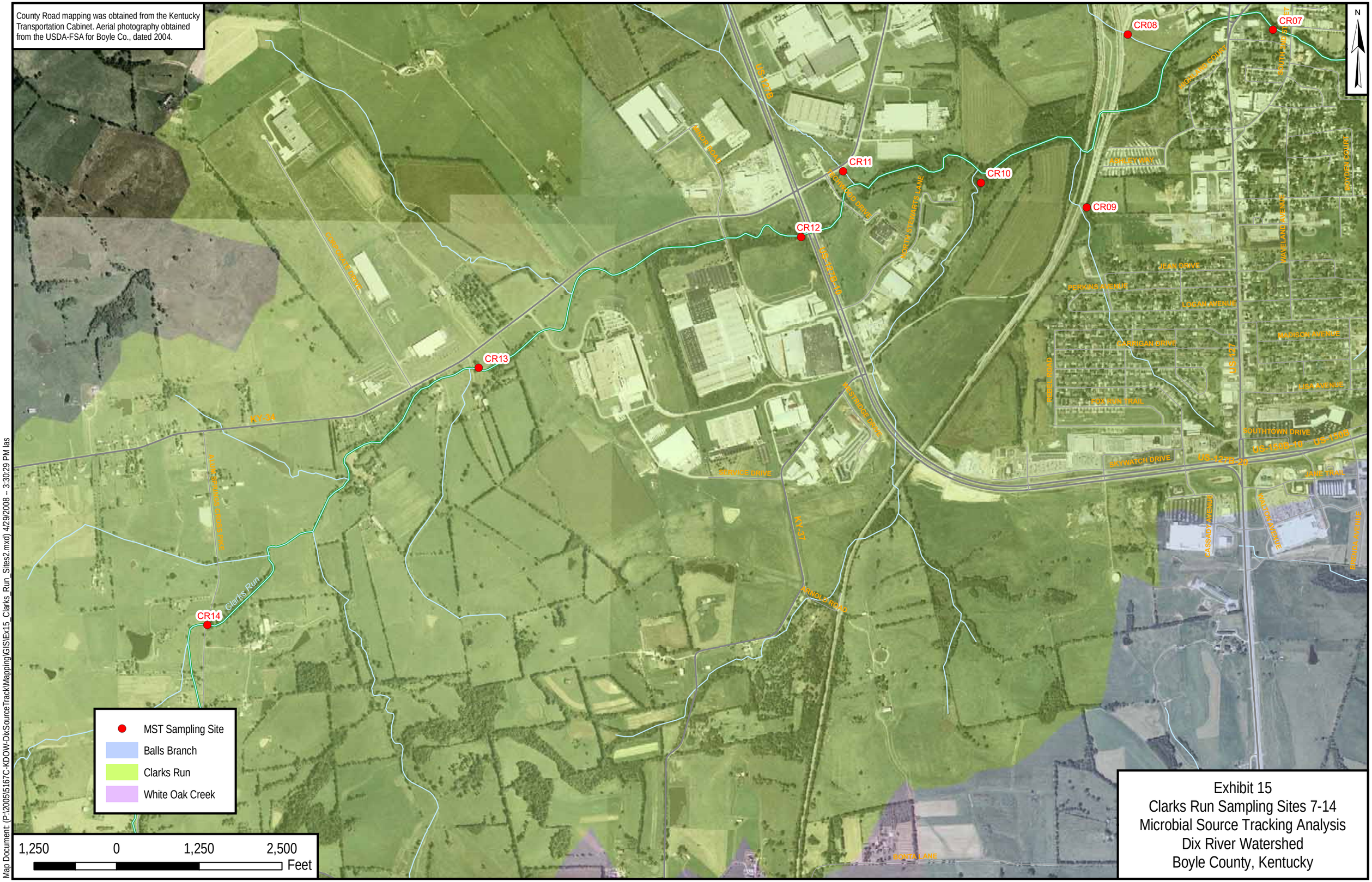


Exhibit 15
Clarks Run Sampling Sites 7-14
Microbial Source Tracking Analysis
Dix River Watershed
Boyle County, Kentucky

Map Document: (P:\2005\5167C-KDOW-DixSourceTrack\Mapping\GIS\Ex15_Clarks_Run_Sites2.mxd) 4/29/2008 -- 3:30:29 PM las

Photographs, GPS waypoints, and flagging of the site location in addition to these field notations are to identify the site. A digital camera, a Garmin GPS or equivalent, tape and spray flagging, chest or hip waders, a Hydrolab multi-probe, a 100-foot tape measure, and a wooden field tape measure will be utilized in this process.

If sample sites must be relocated due to property owner restrictions during the course of work, the site will be relocated downstream of the original location. If downstream relocation is not possible, an upstream location should be chosen and the changes in the land use and potential sources between new location and the original location should be documented. New sites will be given new location point identifications to maintain proper analysis of results.

Characterization of each of these sites will involve completion of Microbial Source Tracking: Site Characterization datasheet and the Watershed Characterization datasheet. In the site characterization, the habitat and land use assessments are representative of a 600-foot reach of the stream over the of the entire sampling period, but the physio-chemical measurements and microbial source characterization are representative of only the sampling time and location at which the were recorded. All parameters except for the physiochemical measurements are considered critical and are absolutely necessary for the completion of the project.

Variability associated with equipment is limited to the water quality probes and measuring devices. Variability associated with these devices can be found in Table 4, page 25. The Hydrolab multi-probe is equipped with four primary sensors, pH, dissolved oxygen, conductivity, and temperature. Turbidity may also be measured on the Hydrolab or by turbidimeter. Source of variability due to climate, flow, or other environmental factors on physiochemical measurements is difficult to quantify.

The Rapid Bioassessment Protocol (RBP) worksheets can be a source of potential variability during physical stream assessment. The intrinsic subjectivity of the physical habitat scoring using the EPA RBP method is a concern for the Dix River Watershed project. To ensure consistency and accuracy with this assessment, Third Rock staff undergoes yearly in-house training that strictly pertains to the EPA RBP scoring protocol. Training methods are based on tutorials provided first-hand to Third Rock by US Army Corps of Engineers (Louisville District). In addition to this training, duplicate measurements will be compared at 10 percent (8) of the sites sampled.

E. coli and Total Coliform Analysis for Hotspot Identification:

E. coli and total coliform analysis for hotspot identification, the second stage of the sampling design, will be conducted at each of the 74 site locations during the Primary Contact Recreation Season (May through October). Two sampling events will be scheduled for this testing, a dry event and a wet event. Samples will be collected an analysis begun within 8 hours of collection. During each event, a Microbial Source Tracking Site Characterization datasheet will be completed and 200 milliliters of water collected from each site. Each sample should be uniquely identified on the sample label and on the accompanying COC. If samples cannot be collected at a station due to dry conditions, the station will not be relocated. A re-sampling event will be scheduled as soon as possible when the water level is adequate for sampling.

Each sample is geographically representative of all the watershed area upstream of the site location. Temporally, the sample represents only the time period during which the sample was collected. The results for *E. coli* and total coliform are applicable only to the volume collected. Ten percent of all sites will also be collected for field duplicates to measure precision. Uncertainty associated with field conditions includes temporal and or transitive

source inputs, temperature, precipitation, the size of the stream, and dynamics of the flow are among some of the uncertainties.

Variability due to sample collection will be minimized by a strict adherence to collection protocols. Consistent field personnel will also reduce variability associated with collection.

In laboratory analysis, multiple controls within the methodology are designed to limit the uncertainty in the results. Limits of this uncertainty are considered in Table 4, page 25.

Analysis of these results will indicate the "hotspots" or geographic areas with the highest concentrations of pathogen input. One primary and one secondary hotspot will be selected from each watershed division (with the exception of the Hanging Fork main stem section from which 2 primary and 2 secondary sites will be selected). Primary hotspot sites will be chosen based on multiple variables including:

- Representativeness of overall watershed area
- Number of upstream sites
- *E. coli* concentration
- Number of potential sources
- Land use

It is expected that many primary sites will often be located at confluences or downstream locations within the watershed areas. The secondary sites will serve as compliments to the primary sites. As such, selection will be more influenced by factors such as concentration, potential sources, and land use. The hotspot sites are at least representative of the area upstream of their location, and may also represent other areas with comparative conditions to a limited degree.

Presence/ Absence PCR and qPCR for Cattle and Human Sources:
Two sampling events will be conducted during the Primary Contact Recreation season to collect MST samples for analysis by Source Molecular, a dry event and a wet event. During the dry event, all primary sites will be sampled, and during the wet event the primary and secondary sites will be sampled as shown in Table 7, on page 48.

Samples are recommended to be shipped to Source Molecular within 72 hours of collection. Four PCR analyses will be performed on each sample to as a quick screening test to determine the presence or absence of the human and cattle markers. Two bacterial species will be utilized as confirmation and differentiation of the source. DNA extracts from samples that test positive for the respective markers will be stored for subsequent level of analysis, qPCR. The qPCR analysis will quantify the markers to gain a better appreciation of the level of fecal pollution from each targeted source. By quantifying the cattle and human inputs into the system, the contribution of sources other than human and cattle may be generally determined by subtraction from the total.

TABLE 7 – MICROBIAL SOURCE TRACKING COLLECTION EVENTS

WATERSHED DIVISION	DRY EVENT COLLECTION SITES	WET EVENT COLLECTION SITES
Within Hanging Fork		
McKinney Branch	1	2
Baughman Branch	1	2
Blue Lick Branch	1	2
Northern unnamed tributary of Hanging Fork	0	0
Frog Branch	1	2
Peyton Branch	1	2
Hanging Fork below West Hustonville	1	2
Hanging Fork main stem and other tributaries	1	2
Within Clarks Run		
Balls Branch West	1	2
Junction City	1	2
Clarks Run	1	2
Total	10	20

For this project, only water samples will be collected and analyzed. Water quality regulations and sampling procedures are framed upon bacterial levels present in the water column. While it has recently been addressed that sediment can be a source of bacterial water quality indicators, the battery of genetic methods employed in the Dix River Watershed can circumvent the ambiguities associated with culture-based methods by discriminating recent from residual fecal pollution. In this initial investigation, the two likeliest sources of fecal contamination, human and cattle, are targeted.

Variability associated with the microbial source tracking methods is discussed in depth in Section 1.5.2.7 of this QAPP.

2.2 Sampling Methods

During all sampling activities, sampling methods and gear utilized is analogous to EPA and KDOW recommendations. All samples collected during this study will be water samples. Specific methods are detailed in the following sections. All samples are to be collected in bottles according to the analytical methods requirements.

Known or suspected deviations from sampling methods, the protocols of this QAPP, or other applicable protocols are to be reported to the Project Administrator. These incidents are documented by email to the project folder and the Project Administrator. All project related emails are to be sent to a central project electronic folder for recall and storage. If the deviation represents a serious flaw with sampling methodology, sampling results, or modeling methods, corrective action will be taken based on recommendations the Project Administrator receives from the KDOW.

2.2.1 Microbial Source Tracking Site Characterization

During site identification and characterization, and in subsequent sampling events the Microbial Source Tracking Site Characterization datasheet should be used to document sources observed in the area. For each site sampled, at minimum the site name and the source category should be documented. If evidence for sources is found, a description of the observation, the location of the source in relation to the site, and estimation of the contribution of this source, GPS points, and photos should be recorded. Source categories are listed at the bottom of the datasheet with some example evidences.

Garmin GPS or the equivalent and a digital camera are necessary to document sources found in the area.

2.2.2 Habitat

During habitat assessment, at the initial and final station visits, a 30-minute minimum visual inspection will be completed at each stream sampling station or reach. Ten habitat parameters will be assessed, according to Methods of Assessing Biological Integrity of Surface Waters in Kentucky (KDOW 2002), including epifaunal substrate (quantity and variety of substrate), embeddedness and pool substrate characterization (measurement of silt accumulation and type and condition of bottom substrate, respectively), velocity/depth regime and pool variability (combination of slow-deep, slow-shallow, fast-deep, and fast-shallow habitats and measurement of the mixture of pool types, respectively), sediment deposition (accumulation in pools), channel flow status (the degree that the channel is filled with water), channel alteration (measurement of large-scale changes in the shape of the channel), frequency of riffles and channel sinuosity (sequence of riffles and meandering of the stream, respectively), bank stability (measure of erosion), bank vegetation (amount of vegetative protection), and riparian vegetative zone width (width of the natural vegetation from the edge of the stream bank through the riparian zone). All of these criteria are rated (1 to 10) and combined to obtain a habitat score (0 to 200) that can be compared to a reference condition. Use attainment can be estimated based on the habitat score.

2.2.3 Physio-chemical Measurements

Temperature, dissolved oxygen, conductivity, turbidity, and pH will be measured during field sampling of the streams with a Hydrolab water quality instrument. Operation of the Hydrolab instrument is conducted in conformance to the Hydrolab operation manual (Hydrolab, 1997). Sampling Technicians shall ensure that the meter is placed in a representative area upstream of sampling disturbance. The meter shall be allowed to equilibrate until no significant change occurs in any parameter over a five second period.

Global Positioning System coordinates will be obtained using a Garmin GPS or the equivalent, accurate to ±5-40m. Readings are measured in NAD83. Internal SOPs and manufacturer's instructions will be followed to record these measurements.

2.2.4 Grab Sample Collection

Samples will be collected directly from the source. When collecting samples, latex gloves will be used to prevent contamination and for the safety of the Sampling Technician. Stream samples will be collected from the thalweg (or

low water channel) just above the stream bottom, upstream of any disturbance to the stream bottom caused by the Sampling Technician. The Sampling Technician will collect the sample by submersing the sample container into the source, taking care not to overfill. Caution will be taken not to scrape the bottom of the source, minimizing excess solids. Proper field data sheets will be completed and field quality controls, as specified in Section 2.5: Quality Control will be collected at this time. Sampling will adhere to the "Kentucky Ambient Watershed Water Quality Monitoring Standard Operating Procedure Manual" for field sampling *e. coli* protocols (KDOW 2005).

During all sampling events, precautions will be taken to ensure the integrity of the collected sample. These tasks include:

- Labeling sample bottles with time and date before filling with water to ensure ink legibility
- Traceable custody shall be documented from the time of sampling until delivered to the laboratory
- Wearing latex gloves during all sampling events to avoid potential sample contamination
- Avoidance of streambed sediment agitation during sample collection
- Immediate placement of sample bottles in ice-filled coolers
- Prompt delivery to laboratory for analysis within the required holding times

2.3 Sample Handling and Custody

2.3.1 Chain-of-Custody

Chain-of-custody (COC) forms will be completed for all samples collected in the field and will follow each sample throughout sample processing. A COC form is a controlled document used to record sample information and ensure that traceability of sample handling and possession is maintained from the time of collection through analysis and final disposition. A sample is considered in custody if it is:

- In the individual's physical possession,
- In the individual's sight,
- Secured in a tamper-proof way by that individual, or secured in an area restricted to authorized personnel

The Sampling and Laboratory Coordinator shall create COCs (as in Appendix D) and provide to the Sampling Technicians. All information shall be documented on the COC in black or blue waterproof permanent ink including field physio-chemical measurements and custody information.

The Sampling Technician shall initiate sample custody at the time the sample is collected. Field custody documentation shall include:

- Verification of Sample Identification
- Number of Sample Bottles Collected
- Collection Date
- Collection Time
- Collector's Signature

The Sampling Technician shall maintain possession of the sample until custody is transferred to the laboratory or another party. The COC shall accompany the sample from the time of collection until it is relinquished. Field

custody is relinquished by signature, with date and time, of the Sampling Technician in the designated area on the COC.

2.3.2 Sample Handling and Transport

The Sampling Technician is responsible for sample handling in the field and transport of samples to the laboratory. He/she will collect the sample at the source following established protocols. He/she is responsible for collecting the sample in appropriately identified collection containers with the correct preservative, as applicable, and ensuring that the container lid is secured tightly to prevent leakage or outside contamination. Sample containers shall be immediately placed in a cooler on ice to maintain a temperature of 4±2° C for transport to the laboratory. Sample bottles shall be placed in the cooler with lid side up in an organized manner per COC entry; this procedure aids the laboratory analysts in preparing samples for analysis within the specified hold time.

Sample coolers should be of adequate size to allow ice to surround all sample bottles. It is the responsibility of the Sampling Technician to ensure that coolers are properly packed in the field and that they have sufficient cooler space on their vehicle for their daily sample load. Coolers shall be secured during transport such that significant disturbance of the samples is avoided.

E. coli and total coliform samples have a holding time of 8 hours. If this hold time requirement is exceeded, the result is qualified and a re-sampling must be scheduled. PCR methodologies do not have hold time requirements, but it is recommended that the samples be delivered to the laboratory within 72 hours of collection.

Upon receipt at the laboratory, the sample custodian shall review the COC for completeness and accuracy. Anomalies shall be documented. The laboratory shall measure sample temperature upon receipt, determine if sample aliquots have been placed in appropriate bottles and properly preserved, and inspect the sample for proper identification and bottle integrity; any discrepancies and/or bottle damage shall be documented on the COC.

2.3.3 Sample Labeling and Identification

Preprinted labels, as shown in Appendix F, indicate Third Rock's name and project identification, and the expected parameters to be analyzed from that bottle. Sampling Technicians are responsible for recording the unique sample identification, as well as the date and time of the collection on each sample bottle. The unique sampling event code follows the following format:

SAMPLE ID = TRC_CC##LP0#-YYYYMMDD

Where:
TRC is the Organization Short Name
CC## is the Station Code. Station Codes consist of the station code (Table 6, page 30) and the unique two-digit number assign to each site within each watershed division (Exhibits 3 through 12)
LP0# is the Location Name. A new location name is established if the sampling site is moved within the same stream reach.
YYYYMMDD is the date in year (YYYY), month (MM), day (DD) format.

In the event that a preprinted label could not be obtained from the laboratory, the Sampling Technician would be responsible for manually recording the information on the sample label. If possible, apply labels and record information before sampling as moisture on the sampling bottles can make adhesion of the label to the bottle difficult.

2.4 Analytical Procedures

Water samples will be analyzed for parameters following the methodology as listed in Appendix C. Modifications to these analytical methods will not be made without the knowledge and consent of Third Rock's Project Administrator.

As current regulations do not specify specific target limits for the analytes involved, the laboratories' internal criteria were cited for this project. The DQIs for each method is recorded in Appendix C. The quantitation limits of individual samples may be raised if a dilution is required to quantify the target compound(s) within the acceptance range.

Source Molecular utilizes internally validated and NELAC approved testing methods for analysis since published standard methods do not exist for the DNA methods used in this project. Summaries of each of these methods can be found in the example sample reports (Appendix E).

In order to properly analyze the parameters associated with the project, the laboratory is required to calibrate and maintain instrumentation and equipment. A list of the key equipment / instrumentation includes:

- Water Incubator
- Autoclave
- Refrigerator
- Centrifuge
- Gradient Thermocycler
- Real-time PCR system
- UV light

The laboratory is required to maintain a corrective action and cause analysis system in order to address deviations and client complaints. When a deviation from an internal procedure or external method or protocol is found or a client has a complaint about the data results or service, the laboratory shall document these incidents and begin a cause analysis to determine the source or sources of the problem. Once the source(s) is/are identified, the laboratory shall institute corrective action to achieve compliance. Evidence of completion of this corrective action and follow up evaluation of the effectiveness of the action, as necessary, shall demonstrate compliance.

Samples are disposed of by pouring the neutralized sample into a conventional drain to the municipal sewage treatment system. Due to the hold times on total coliform samples, samples for total coliform may be disposed of after the completion of the analysis. For DNA samples, the laboratory shall maintain the sample until the completion of the project. Bacterial cultures or growth media produced during the analysis are to be autoclaved prior to disposal.

It is the expectation of Third Rock that laboratory results are delivered by Source Molecular within 11 business days of sample receipt.

2.5 Quality Control

2.5.1 *E. coli* and Total Coliform

To ensure that data of known and documented quality are generated in the sampling and analysis of *E. coli* and total coliform, the QC criteria described in this section must be met. The QA Manager is responsible for assuring proper adherence to these procedures for Third Rock's laboratory analysis.

- Field Duplicate Sample

Approximately 5 percent of all samples taken in the field are duplicated. To perform a field duplicate, the Sampling Technician shall consecutively collect 2 representative aliquots, independent of one another, from the same source by the grab collection technique.

- Duplicates

A laboratory duplicate sample (DUP) is prepared at a frequency of 1 per 20 field samples at minimum. The relative percent difference (RPD) between duplicate samples for samples having analyte concentrations greater than their respective reporting limit must be within the acceptance ranges. If the QC criteria for duplicate sample are not satisfied, the cause of the problem must be determined and corrected. If the problem adversely affected the entire analysis batch, all samples in the batch must be reanalyzed.

- Sterility Check

At the beginning of each filtration series and after every 10 samples, conduct a sterility check on the media, filters, and the filtration apparatus used in testing. If growth occurs, this may indicate contamination of the media, filtration apparatus, filters, or petri dishes. Alternatively, growth may indicate poor aseptic technique. Before disposing of the media, filters, or petri dishes, repeat sterility check using proper aseptic technique. Also attempt to isolate sources of contamination. If growth occurs again, isolate the source of contamination. No testing of samples can be performed until the source of contamination is discovered and eliminated. All samples performed concurrently with the sterility check may be compromised and the testing should be repeated with new samples. All data is recorded in the appropriate logbook.

- Incubator Temperature Check

Twice daily on days when the incubator is in use, the temperature of the incubator is to be recorded in the incubator temperature logbook. Readings must be made at least 4 hours apart on each of the days of use. If readings are outside of the 35 ± 0.5^{o} C window, test results are considered invalid and samples must be recollected. The incubator is to be adjusted and remain within the criteria for 4 hours before use for reanalysis.

- Confirmation Check

One of every 20 positive samples is confirmed using an Enterotube II confirmation. To perform the confirmation, the manufacturers' directions are followed and the results recorded on the appropriate datasheet.

- Positive Control Check

On a monthly basis (or if sample collection is less frequent than monthly prior to a run), a positive control check of the media is performed. A suspension is prepared by transferring a bacterial growth from a culture plate. The suspension is used as a sample following the above procedures. Results indicate the presence of typical colonies or growth. Data is recorded in the appropriate logbook.

- Analyst Comparative Count Check

On one run per month, analysts independently count the number of *E. coli* and coliforms observed. Results are to be recorded on the appropriate datasheet.

2.5.2 PCR and qPCR

Source Molecular utilizes the quality controls specified in the Quality Policy Manual (Appendix C) and the Microbiology Guide Document and Quality Control Procedures (Appendix G) to ensure that data is of sufficient quality for use. These documents indicate the frequency, criteria, and corrective action specific to the PCR and qPCR techniques (page 9 and 10 of Microbiology Guide Document; pages 55 through 58 of the Quality Policy Manual) that are used in this project.

In general, quality control is maintained by the use of internal positive and negative controls for presence/absence tests and by running DNA standards of known quantity with each qPCR run. Thresholds and baselines are calculated with computer software and all results are confirmed manually after each run. Five separate DNA standards, each with a known quantity of target DNA are run in triplicate with every qPCR run. Deviations of greater than 10 percent between runs will trigger a data flag.

2.5.3 Calculations

The following calculations are used in the interpretation of the data provided by the quality controls:

- Accuracy

For QC samples of known concentration, accuracy is quantified by calculating the *percent recovery* (%R) of analyte from a known quantity of analyte as follows:

$$\%R = \frac{V_m}{V_t} \times 100$$

where:

V_m = measured value (concentration determined by analysis)
V_t = true value (concentration or quantity as calculated or certified by the manufacturer)

- Precision

When calculated for duplicate sample analyses, precision is expressed as the *relative percent difference* (RPD), which is calculated as:

$$RPD\ (\%) = \frac{|S - D|}{(S + D)/2} \times 100$$

where:
S = first sample value (original result)
D = second sample value (duplicate result)

2.6 Instrument/Equipment Maintenance and Calibration

All sampling equipment will be maintained and calibrated according to manufacturer recommendation.

The Hydrolab runs on battery power and thus the charge must be maintained by charging on a daily basis. Calibration shall be completed in accordance with the user manual (Hydrolab, 1997) on a weekly basis.

All field and laboratory supplies are acquired through Third Rock's vendors. The members on this vendor list have applied quality control measures that have resulted in recurring quality.

All maintenance on laboratory equipment is conducted in accordance with manufacturers' recommendations. These requirements are described in the laboratories' standard operating procedures and appropriate instrument maintenance manuals. The applicable laboratory is responsible for ensuring that timely maintenance is conducted and that sufficient spare parts are on hand for necessary maintenance and repair procedures.

The frequency of maintenance performed depends on the equipment; laboratory maintenance is scheduled and conducted daily, monthly, weekly, quarterly, semiannually, and annually, as required. A few maintenance needs (*e.g.*, accidental breakage, part failure) are not covered by the general maintenance schedule, and such maintenance is performed as needed.

Specific instrument calibration requirements can and do vary slightly depending on the particular method and the project and regulatory requirements for the project. Detailed descriptions of specific calibration requirements are provided in the laboratory analytical method SOP for each method.

2.7 Non-Direct Measurements

Non-direct measurements include any measurements or data that will be used during this project that will not be directly measured by Third Rock or Source Molecular. The Lincoln and Boyle County health department files on onsite treatment will be utilized to establish the GIS data set. Data must meet the specifications listed in Table 2, page 16, for use. Access to this key resource is expected to be provided by the health departments. Aerial and topographical maps will also be utilized in the construction of GIS maps.

2.8 Data Management

Records are to be stored until 3 years after the close of the project. An efficient and effective data management system is necessary to maintain and store all project related data.

The laboratory is expected to maintain all records associated with the analytical results, including laboratory notebooks, bench sheets, instrument calibration and sequence logs, preparation logs, maintenance logs, etc., for the retention period of the grant according to their internal data management procedures.

All field and laboratory data and results will be reviewed, organized, and stored by Third Rock's Laboratory and Sampling Coordinator. In order to accomplish this task, the Sampling Technician shall submit completed field datasheets and copies of measurements in field notebooks to the Sampling Coordinator upon return to the office.

The Laboratory and Sampling Coordinator will review the datasheets for completeness. If the Sampling Technician submits samples to the laboratory, he/she shall obtain a copy of the relinquished COC and submit it to the Sampling Coordinator. If the sampling technician relinquishes the COC to the Sampling Coordinator, the Sampling Coordinator shall similarly obtain a copy of the relinquished COC to retain for recording purposes.

The Laboratory and Sampling Coordinator stores the field data and the COC until results are received from the analytical laboratory. She then submits the hardcopy results to the QA Manager for quality evaluation. Once the review is completed, the files are returned to the Laboratory and Sampling Coordinator who organizes and stores the hardcopies of all information in the designated project folder in the central files.

Third Rock will also deliver analytical data in a COMPASS format to the KDOW per event completion for all sampled stations. The Data Manager will compile the field and laboratory results into the required COMPASS template for submission. Once all data entry is completed, he will perform a data verification. Using a custom designed verification program within the Access data entry template, a report is generated showing deviations in the COMPASS template. Each deviation is documented and investigated by the Data Manager. Once the verification is complete, the Data Manager forwards the files to the QA Manager for review. Once the review is completed, the QA Manager notifies the Project Administrator who submits the file to the KDOW.

All project related correspondence is documented by an email system. All project related emails are copied to the Third Rock assigned project file folder for traceability and storage. All other electronic files are stored on a central project drive accessible to the appropriate Third Rock personnel.

3 Assessment and Oversight

3.1 Assessment and Response Actions

Assessment and response actions are necessary to ensure that this QAPP is being implemented as approved. For a general summary of these assessments see Table 8, page 57. The Kentucky Division of Water (KDOW) quality assurance officer (QAO) may freely review all field and laboratory techniques as requested. Any identified problems will be corrected based on recommendations by the QAO. The KDOW will also review analytical results on a monthly basis.

TABLE 8 – MICROBIOLOGY GUIDE DOCUMENT AND QUALITY CONTROL PROCEDURES

ASSESSMENT TYPE	FREQUENCY	PURPOSE	INTERNAL OR EXTERNAL	PARTIES RESPONSIBLE FOR PERFORMING		METHOD OF REPORTING
				PERFORMING ASSESSMENTS	RESPONDING TO ASSESSMENTS	
KDOW Audit	As requested	Ensure conformance to project objectives	External	KDOW	Parties of concern	Corrective Action Response
Laboratory Demonstration of Capability	Prior to initial analysis	Ensure analyst is capable of performing the method to specifications.	Internal	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Laboratory Internal Audits	Annually, at minimum	Ensure conformance to methods, regulations, and procedures.	Internal	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Laboratory External Audits	Usually biannually	Ensure conformance to methods, regulations, and procedures.	External	Regulatory Body	Laboratory QA Director	Internal Lab documentation
Project Status Meeting	Monthly, as needed	Evaluate the status on project related objectives and concerns	Internal	QA Manager	Project Administrator	Status Meeting Minutes
Field Systems Audit	Once per Project	Assess sampling technicians adherence to proper documentation and protocols.	Internal	QA Manager	Sampling Technicians	Email Correspondance
Field Data Review	Each Sampling Event	Review for completeness and accuracy in COCs and field sampling datasheets.	Internal	Sampling Coordinator	Sampling Technicians	Verbal or Email documentation
Analytical Results Review	Monthly	Assess progress and results of analytical findings of each station.	External	KDOW	Project Administrator	Analytical Monthly Summary
COMPASS Verification	Per Data submission	Ensure data entry is correctly transcribed and the format corresponds to specifications	Internal	Data Manager	KDOW	Internal Verification Report
Quality Evaluation	Upon Completion of the Data	Provide overall assessment of the quality of the data.	Internal	QA Manager	KDOW	QA Evaluation

3.1.1 Laboratory Assessments

To ensure conformance with this QAPP and the applicable regulations, certifications, and methods by which the laboratories operates, the laboratory performs several assessment measures. To ensure that the analyst is capable of performing the requested analytical methods to specifications, each analyst is required to acceptably demonstrate this ability prior to conducting sample analyses. The analyst must conduct four replicate analyses of a known standard and achieve precision and accuracy equal to or better than the acceptance ranges for laboratory duplicates and laboratory control samples, respectively.

The laboratories are also required to have routinely scheduled internal and external audits. The laboratory QA Director or their appointee on an annual basis performs internal audits. Certification bodies usually on a biannual basis perform external audits. In each case, the findings of the audit, both positive and negative are documented, and the corrective response to the cited deviations is required within thirty days of receipt of the audit report. Corrective actions are submitted to the auditing body for review and approval. When findings cast doubt on operations or validity of results, the laboratory takes timely corrective action, and notifies clients in writing within 30 days if investigations show results may have been affected, such as through identification of defective measuring or test equipment.

3.1.2 Field Assessments

The QA Manager is responsible for the overall conformance of Third Rock to the general procedures, protocols, and methods established by this QAPP and internal project related procedures. To ensure overall conformance to this QAPP, the QA Manager schedules and manages a status meeting monthly, or as needed for this project. At this meeting, the status of progress on project related objectives is discussed and concerns addressed. The Project Administrator is responsible for compiling the minutes of these meetings for review by the QA Manager. These minutes are stored electronically in the project files. The QA Manager may apply spot assessments including supervision of field activities and documentation thereof including calibration and maintenance of field equipment or sample collection techniques. Deviations found in such assessments are reported to the Project Administrator and the Sampling Technicians and documented in the project email files. The QA Manager may also periodically review the project correspondence files to ensure that all deviations are properly documented and resolved.

The Sampling Coordinator will supervise and assess the Sampling Technicians for conformance on field measurements and documentation and sample handling and custody documentation. The Sampling Coordinator will document significant deviations in project emails to the Sampling Technicians and the Project Administrator. Minor deviations, such as typos or missing dates, may be communicated verbally. All corrective actions will similarly documented by email correspondence in the project file.

3.2 Reports to Management

Third Rock will prepare a final report that will describe all methods and findings of this project. The final report will satisfy all requirements for the grant. Involved in that final report will be a Quality Assurance Evaluation assessing the quality of the data produced.

Prior to the completion of that report, reports on the progress and assessment of the project objectives are produced as summarized in Table 8, page 57. All reports are expected to list the personnel or organization responsible for producing the report and the date prepared for traceability purposes.

4 Data Validation and Usability

4.1 Data Review, Verification, and Validation

Data review is the internal examination to check if data has been recorded, transmitted, and processed correctly. Data verification is the process of evaluating whether the data meets method, procedural, or contractual specifications. Data validation is the review of the quality of the data based on the specific DQIs indicated in this QAPP.

The Sampling Technician performs data review for all field data initially, and the Sampling Coordinator subsequently reviews this data. For the laboratory work, the laboratory analyst initially conducts the review, and the data is peer reviewed by another analyst or capable reviewer. Data is reviewed for data entry, calculations, and transformations as well review of quality control criteria. If deviations are noted, corrective actions are taken with verification of both the reviewer and the original data collector. If consensus cannot be reached, the data is rejected.

During verification and validation of the data, all data that does not meet the DQIs (listed in Table 4, page 25) listed in this QAPP will be qualified or rejected. Any qualified data points will be further analyzed by the QA Manager to evaluate whether the data should be used under the qualifications or rejected. If the nature of the qualification is such that the result may be significantly impacted, the result will be rejected.

4.2 Verification and Validation Methods

Responsibility for verification and validation of the project requirements is divided based on the roles of the key personnel and the elements of the project they are involved with.

The Project Administrator is responsible for ensuring all contractual requirements of the Dix River Watershed MST project are met. In order to ensure these objectives are met according to KDOW standards, the Project Administrator verifies project progress against the tasks listed in the contract and this QAPP. This verification is conducted by project meetings and other communications with key personnel on the progress that has been made and its conformance to project specifications. Deviations from project specifications are to be reported to the Project Administrator.

Aiding the Project Administrator, the QA Manager is responsible for data validation for this project. Data validation is accomplished by comparison of the DQIs listed in this QAPP with the data that is produced during the course of the project. All field and laboratory data are to be reviewed by the QA Manager who produces a QA Evaluation of the data. This review, performed within one week of receipt of the results, assesses the completeness and accuracy of the data and investigations any qualified data.

The Sampling and Laboratory Coordinator is responsible for verifying the completeness and accuracy of all field data sheets, COCs, and laboratory reports. She will document significant deviations in project emails to the Sampling Technicians, Laboratories, and the Project Administrator. Minor deviations, such as typos or missing dates, may be communicated verbally. All corrective actions will similarly documented by email correspondence in the project file.

The Laboratory Quality Manager is responsible for the validation and verification of all laboratory methods. Through audits, peer review, supervision, and method validation and other techniques, the Laboratory Quality Manager ensure that method specifications are properly followed. Additionally the Laboratory Quality Manager is responsible to ensure peer review of manipulation of all data (including data entry, calculations, and transformations as well review of quality control criteria) is conducted on the data. Corrective actions are taken, if possible, while the samples are still within the method specified holding time. Data quality flags are applied to the laboratory results that do not meet these requirements.

The Data Manager is responsible for verifying that the electric data meets the COMPASS requirements and that the customized COMPASS software is functioning properly. To ensure accurate data entry for into COMPASS, all entries and calculations are verified by an independent review and through custom verification programs. The COMPASS verification program verifies that all required data fields are completed, all lookup values are valid, analytical results are correctly formatted, sampling dates correspond to sample IDs, verifies location points, and performs miscellaneous formatting checks. Multiple test runs evaluate the proper functioning of this software ensuring that the proper changes are being made or flagged for review. The COMPASS verification tool produces an internal report of any discrepancies in the data to be resolved by the Data Manager in conjunction with the QA Manager. All COMPASS data is further screened by the KDOW for conformance to their internal specifications before acceptance.

4.3 Reconciliation with User Requirements

Associated with the final report will be a QA Evaluation Report. This report will consider all aspects of the quality of the data and will address the uncertainty involved in the data. Because of the nature of the sampling design, quantitative estimates of the applicability of the data are difficult because the data was gathered under a judgment based design process. A description of the data and the limits of uncertainty of the data due to field and laboratory accumulation will be discussed. All qualified data will be investigated and limitations on its use will be discussed. Qualitative statements about the data and its applicability will be made in the final report.

5 References

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Assoc., American Water Works Assoc., and Water Pollution Control Federation. 20th Edition. Washington, DC.

Brion, G.M. and Mao, H.H. 2000. *Use of Total Coliform Test for Watershed Monitoring with Respects to Atypicals*. ASCE Journal of Environmental Engineering, Vol. 125, No. 2, 175-181.

Brion, G.M., J.S. Meschke, and M.D. Sobsey. 2002. *F-Specific RNA Coliphages: Occurrence, Types, and Survival in Natural Waters.* Water Research 36:2419-2425.

Brion, G.M. 2005. *Application of the AC/TC Bacterial Ratio for Watershed Quality Management of Stormwater Introduced Pathogens.* ASCE Conf. Proc. 173, 295

Colford JM Jr, Wade TJ, Schiff KC, Wright CC, Griffith JF, Sandhu SK, Burns S, Sobsey M, Lovelace G, Weisberg SB. 2007. *Water Quality Indicators and the Risk of Illness at Beaches with Nonpoint Sources of Fecal Contamination.* Epidemiology. 18: 27-35.

Cumberland Environmental Group, LLC. 2005. Peyton Creek Frog Branch Data Report: pre-BMP Monitoring Analysis. Henryville, IN.

Field, K.G. etal. 2003. *A Comparative Study of Culture-Independent, Library-Independent Genotypic Methods of Fecal Source Tracking.* J. Water Health 1: 181-194

Field, K. G. and T.M. Scott. 2007. Microbial Source Tracking. Background paper for Source Tracking workshop at Michigan State University, March 17, 2007. http://cws.msu.edu/documents/MicrobialSourceTrackingWhitePaper.pdf

Hydrolab Corporation. 1997. Surveyor 4 Water Quality Data Display User's Manual. Austin, TX.

Jenkins, T.M., T.M. Scott, M.R. Morgan, and J.B. Rose. 2005. *Occurrence of Alternative Fecal Indicators and Enteric Viruses In Michigan Rivers*. J. Great Lakes Res. 31: 22-3.

Kentucky Division of Water. 2005. 2004 303(d) List of Waters for Kentucky. Natural Resources and Environmental Protection Cabinet, Water Quality Branch. Frankfort, Kentucky.

Kentucky Division of Water. 2002b. Methods for Assessing Biological Integrity of Surface Waters in Kentucky. Natural Resources and Environmental Protection Cabinet, Water Quality Branch. Frankfort, Kentucky.

Kentucky Division of Water. 2005. Kentucky Ambient Watershed Water Quality Monitoring Standard Operating Procedure Manual. Environmental and Public Protection Cabinet. Department for Environmental Protection. Frankfort, Kentucky.

Liu L, Phanikumar MS, Molloy SL, Whitman RL, Shively DA, Nevers MB, Schwab DJ, Rose JB. 2006. *Modeling the Transport and Inactivation of E. Coli and Enterococci in the Near-Shore Region of Lake Michigan*. Environ Sci Technol. 40:5022-8.

McQuaig, S.M., T.M. Scott, V.J. Harwood, S.R. Farrah, and J. Lukasik. 2006. A Novel Method for the Detection of Human Derived Fecal Pollution in Environmental Waters Using a PCR Based Human Polyomavirus Assay. Appl. Environ. Microbiol. 72:7567-74.

Jenkins, T.M., T.M. Scott, M.R. Morgan, and J.B. Rose. 2005. *Occurrence of Alternative Fecal Indicators and Enteric Viruses In Michigan Rivers*. J. Great Lakes Res. 31: 22-3.

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.

Shanks OC, Nietch C, Simonich M, Younger M, Reynolds D, Field KG. 2006a. *Basin-Wide Analysis of the Dynamics of Fecal Contamination and Fecal Source Identification In Tillamook Bay, Oregon*. Appl Environ Microbiol. 72: 537-46.

Shanks OC, Santo Domingo JW, Lamendella R, Kelty CA, Graham JE. 2006b. *Competitive Metagenomic DNA Hybridization Identifies Host-Specific Microbial Genetic Markers in Cow Fecal Samples.* Appl Environ Microbiol. 72: 4054-60.

Soule M, Kuhn E, Loge F, Gay J, Call DR. 2006. Using DNA *Microarrays to Identify Library-Independent Markers for Bacterial Source Tracking.* Appl Environ Microbiol. ;72:1843-51.

Source Molecular Corporation. 2005. Quality Policy Manual. Miami, FL.

U.S. Environmental Protection Agency (USEPA). 1979. Methods for Chemical Analysis of Water and Wastes. Environ. Monit. and Support Lab. Las Vegas, Nevada. EPA/600/4-85/048.

USEPA. 1986. Ambient Water Quality Criteria for Bacteria – 1986. Office of Water Regulations and Standards Criteria and Standards Division. Washington, DC. EPA440/5-84-002.

USEPA. 2005a. Handbook for Developing Watershed Plans to Restore and Protect Our Waters. Office of Water: Nonpoint Source Control Branch. Washington DC. EPA 841-B-05-005.

USEPA. 2005b. *Microbial Source Tracking Guide Document*. Office of Research and Development, Washington, DC. EPA 600/R-05/064. 131pp.

U. S. Geological Survey (USGS). 2000. Modeling Hydrodynamics and Water Quality in Herrington Lake, Kentucky. Water-Resources Investigations Report 99-4281

Wade TJ, Calderon RL, Sams E, Beach M, Brenner KP, Williams AH, Dufour AP. 2006. *Rapidly Measured Indicators Of Recreational Water Quality Are Predictive of Swimming-Associated Gastrointestinal Illness*. Environ Health Perspect ;114:24-8.

Wade TJ, Pai N, Eisenberg JN, Colford JM Jr. 2003. *Do U.S. Environmental Protection Agency Water Quality Guidelines For Recreational Waters Prevent Gastrointestinal Illness? A Systematic Review and Meta-Analysis*. Environ Health Perspect.111:1102-9.

APPENDICES

APPENDIX A – MICROBIAL SOURCE TRACKING SITE CHARACTERIZATION DATASHEET

Microbial Source Tracking: Site Investigation Data Sheet

Project #:	Date: / / Time: ☐AM ☐PM	Investigator(s):

Site Name	Source Category*	Description of Observation and Location	Distance (ft) and Orientation[+] to Site Location	Estimation of Contribution	GPS Coordinates (DMS) or Label	Photo(s)

*Source Categories include: H=Human (sewer, septic tank, straight pipe, etc.), L=Livestock (horses, cattle, pigs, etc.), P=Domestic Pets (cats, dogs, etc.), A=Avian (geese, ducks, etc.), W=Other Wildlife (deer, muskrat, raccoon, etc), N/A = None Observed

[+] Orientation: U= Upstream, D= Downstream. Site investigation should be conducted at least 300ft upstream and downstream of sampling location.

APPENDIX B – WATERSHED CHARACTERIZATION DATASHEET

STREAM NAME:	LOCATION:
STATION #: MILE:	BASIN/WATERSHED:
LAT.: LONG.:	COUNTY: USGS 7.5 TOPO:
DATE: TIME: ☐AM ☐PM	INVESTIGATORS:

TYPE SAMPLE: θ P-CHEM θ Macroinvertebrate θ FISH θ BACT. θ ALGAE

WEATHER:

Now	Past 24 hours
θ	θ Heavy rain
θ	θ Steady rain
θ	θ Intermittent showers
θ	θ Clear/sunny

Has there been a heavy rain in the last 7 days?
θ Yes θ No
Air Temperature _____ °C. Inches rainfall in past 24 hours _____ in.
_____ % Cloud Cover

P-Chem: Temp(°C)_____ D.O. (mg/l)_____ %Saturation_____ pH(S.U.)_____ Cond._____ θ Grab

INSTREAM WATERSHED FEATURES:

Stream Width	_____ ft
Range of Depth	_____ ft
Average Velocity	_____ ft/s
Discharge	_____ cfs
Est. Reach Length	_____

Check the categories that best describe the general appearance of the stream:

Litter:
. No litter visible
. Small litter occasionally (e.g., cans, paper)
. Small litter common
. Large litter occasionally (e.g., tires, carts)
. Large litter common

Streambank Erosion:
. No erosion or areas of erosion very rare; no artificial stabilization
. Occasional erosion
. Areas of erosion common
. Artificial stabilization (e.g., rip rap)

Special Problems (note in detail in comment section below):
. Spills of chemicals, oil, etc.
. Fish kills
. Wildlife, waterfowl kills
. Flooding
. Periods of no flow

Note the number of hydrologic modifications (structures that alter flow):
None _____ Waterfalls _____
Dams_____ Stream Fords _____
Bridges_____ Beaver dams_____

Stream Flow:
θ Dry θ Pooled θ Low θ Normal
θ High θ Very Rapid or Torrential

Stream Type:
θ Perennial θ Intermittent
θ Ephemeral θ Seep

Riparian Vegetation: Dom. Tree/Shrub Taxa
Dominate Type:
θ Trees θ Shrubs
θ Grasses θ Herbaceous
Number of strata ____

Canopy Cover:
θ Fully Exposed (0-25%)
θ Partially Exposed (25-50%)
θ Partially Shaded (50-75%)
θ Fully Shaded (75-100%)

Note the approximate length of stream that is affected by the following:
Stream diversion_____
Stream straightening_____
Concrete streambank/bottom_____

Substrate θEst. θP.C.	**Riffle_____%**	**Run_____%**	**Pool_____%**
Silt/Clay (<0.06 mm)			
Sand (0.06 – 2 mm)			
Gravel (2-64 mm)			
Cobble (64 – 256 mm)			
Boulders (>256 mm)			
Bedrock			

Habitat	**Condition Category**			
Parameter	**Optimal**	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Sow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr.) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8.Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score **NOTES/COMMENTS:**

LAND USES IN THE WATERSHED

1. Specific uses identified (check as many as apply)

	Streamside	100—200 Yards
Residential:		
Single-family housing	.	. .
Apartment building	.	. .
Lawns	.	. .
Playground	.	. .
Parking lot	.	. .
Other _____	.	. .
Commercial / Industrial / Institutional:		
Commercial development	.	. .
(stores, restaurants)	.	. .
Auto repair/gas station	.	. .
Factory/Power plant	.	. .
Sewage treatment facility	.	. .
Water treatment facility	.	. .
Institution (e.g., school, offices)	.	. .
Landfill	.	. .
Automobile graveyard	.	. .
Bus or taxi depot	.	. .
Other _____	.	. .
Forest / Parkland:		
Recreational park	.	. .
National/State Forest	.	. .
Woods/Greenway	.	. .
Other _____	.	. .
Agricultural / Rural:		
Grazing land	.	. .
Cropland	.	. .
Animal feedlot	.	. .
Isolated farm	.	. .
Old (abandoned) field	.	. .
Fish hatchery	.	. .
Tree farm	.	. .
Other _____	.	. .

Summary of major land uses in the watershed (use approx. percentages)

Residential ____%
Parkland/Forest ____%
Commercial/Industrial/Institutional ____%
Other ____%
Agricultural/Rural ____%

3. Additional activities in the watershed (check as many as apply)

	Streamside	100—200 Yards
Construction	.	. .
Building construction	.	. .
Roadway	.	. .
Bridge construction	.	. .
Other _____	.	. .
Logging		
Selective logging	.	. .
Intensive logging	.	. .
Lumber treatment facility	.	. .
Other _____	.	. .
Mining		
Strip mining	.	. .
Pit mining	.	. .
Abandoned mine	.	. .
Quarry	.	. .
Other _____	.	. .
Recreation		
Biking/Off-road vehicle trails	.	. .
Horseback riding trail	.	. .
Boat ramp	.	. .
Jogging paths/hiking trail	.	. .
Swimming area	.	. .
Fishing area	.	. .
Picnic area	.	. .
Golf course	.	. .
Campground/trailer park	.	. .
Power boating	.	. .
Other _____	.	. .

4. Comments on land uses

Use this space to explain or expand on land use descriptions you have identified above. For example, you might want to identify particular buildings, specify the location of construction sites, note the condition streamside picnic areas, note the presence of cows in a stream, or note corrective measures such as swales or settling basins. **Provide information on pipes and drainage ditches found on the banks or in the stream. Identify type of pipe** (Industrial outfall, sewage treatment plant outfall, storm drain, combined sewer overflow, agricultural field drainage, paddock or feedlot drainage, settlement basin/pond drainage, parking lot drainage, etc.).

APPENDIX C – SOURCE MOLECULAR QUALITY POLICY MANUAL

EPA ID# FL01249

Quality Policy Manual (QPM-001a)

Use with Laboratory Microbiology Guide Document
and Quality Control Procedures (MGD-1)

Laboratory Directors:

Thierry Sam Tamers
Chief Executive Officer
8370 SW 100th Street
Miami, FL 33156
(305) 595-7386

Troy M. Scott, Ph.D.
Technical Director, QA Officer
9460 SW 227th Lane
Miami, FL 33190
(352) 317-5713

Source Molecular Corporation
4842 SW 74th Court
Miami, FL 33155

Distribution Table

Document Number QPM-001

Copy Number _____

Revision: 2

Revision Date: 12/05

This controlled copy is issued to: _____

Date issued:_____

Quality Policy Manual
Information Page

Document Title:	Quality Policy Manual
Document Number:	QPM-001
Issuer:	Quality Assurance
Approved By:	_____ Thierry Sam Tamers
	_____ Troy M. Scott, Ph.D.
Organization:	Source Molecular Corporation
Address:	4842 SW 74th Court Miami, FL 33155

Department Approvals

Laboratory Director: _____Date: _____
Thierry Sam Tamers

Technical Director: _____Date: _____
Troy M. Scott, Ph.D., QA Officer

Table of Contents

Quality Policy Manual — 1. Introduction

Introduction

The Source Molecular Corporation is pleased to approve the Quality System Manual in compliance with NELAP Standards (2003). The Lab Directors and upper management of the Source Molecular Corporation support fully this whole quality system as described herein.

Organization Overview

The name of this organization is the Source Molecular Corporation. It is located at 4842 SW 74th Court, Miami, FL 33155

The company provides testing services for microbiological samples.

The company was founded in 2002 and employs 2 people.

Mission

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.

Quality Policy Manual — 2. Quality Policy and Objectives

Policy Statement

The management of the Source Molecular Corporation is committed to good professional practice and to provide analytical services in compliance with stringent standards of quality. All analyses performed by Source Molecular Corp. shall be in accordance with established assurance practices and specific, written testing procedures. All employees shall be familiar with their responsibilities under the program and implement the policies and procedures in their work. The quality manual shall be readily available to all employees and maintained up-to-date along with quality documentation.

Objectives

1. Test results shall be of known quality
2. The precision and accuracy of all test data shall be determined
3. Data acquisition, transfer and report preparation steps shall be documented
4. All reports shall be reviewed for completeness and conformance to the quality system program by the appropriate department head, the lab director or the lab manager.
5. Raw data, quality control data and reports shall be stored and retrievable
6. Sample receiving shall ensure that Source Molecular Corporation sample acceptance policy is met.
7. Samples shall be retrievable until disposal is called for
8. All operations shall be performed in accordance with and in conformance to detailed, documented standard operating procedures.

Test Methods for which Accredited Testing is Being Performed

EPA Method 1600 – Enterococci in Water by membrane filtration and mEI agar - Pending
EPA Method 1604 – E. coli in water by membrane filtration and mI agar - Pending

Organization Structure

Source Molecular Corp. operates its testing facilities at one location at 4842 SW 74th Court, Miami, FL 33155. It shares premises with BCS Laboratories, Inc. and the EnviroGenetics Corporation.

The senior executives responsible for operation are the Lab Directors.

Source Molecular Corporation maintains a written organization chart designating positions and responsibilities of other company officers, managers as shown in the organization chart.

Approved Signatures for Laboratory

All documents bearing SOURCE MOLECULAR CORPORATION letterhead or those which are directly related to business conducted by Source Molecular Corporation, including official reports, legal documents, etc. must be signed by Dr. Troy M. Scott or Thierry Sam Tamers. Documents bearing any other names will not be considered as legal or certified documents.

Organization and Management Structure

Lab Director – Thierry Sam Tamers
Lab Director/QA Officer - Troy M. Scott, Ph.D.

Relationship between Management, Support Services, and Quality System

Dr. Troy Scott serves as director and principal investigator of the laboratory. Dr. Scott is also the laboratory Quality Manager and Technical Director and is responsible for implementing and enforcing standard operating procedures as well as maintaining all calibration and monitoring logs (see 4.1.5.h). Thierry Sam Tamers is owner and Chief Operating Officer of the Source Molecular Corporation

Job Descriptions of Key Staff

Thierry Sam Tamers – Mr. Tamers serves as Chief Operating Officer of the Corporation. Responsibilities include client interaction, reporting, and quality control of all released documents.

Troy M. Scott, Ph.D. – Laboratory Director/Quality Manager – Dr. Scott oversees and validates final results and implements and enforces all quality assurance/quality control measures. Furthermore, Dr. Scott supervises field collection and processing of samples, and directs the environmental virology, parasitology, and molecular biology divisions of the company. Finally, Dr. Scott maintains the corporation's current Quality Manual, oversees and validates final results, prepares final reports, and supervises or conducts all analyses.

Policy

Executive management of Source Molecular Corp. is responsible for establishing Policy, Objectives, and Commitment to quality.

Quality System and Management Requirements (NELAC Manual Chapter 5.4 – 5.4.1.5)

4.1.1 Source Molecular Corporation is an entity that can be held legally responsible.

4.1.2 Source Molecular Corporation conducts activities to be compliant with NELAP Standards, the needs of the client, regulators, or recognition bodies.

4.1.3 The management system covers all work carried out in the laboratory's permanent location.

4.1.4 The organizational structure of Source Molecular Corporation is defined in order to identify potential conflicts of interest. Source Molecular Corporation does not perform activities other than environmental testing.

4.1.5 The laboratory shall:

a) Provide personnel with the authority and resources to carry out their duties and to identify the occurrence of departures from the quality system or from the procedures for performing environmental tests, and to initiate actions to prevent or minimize such departures.

b) Have processes to assure that staff is free from undue internal and external pressures and influences that may adversely affect the quality of their work.

c) Protect the client's confidential information and proprietary rights, including the electronic storage and transmission of results.

d) Avoid involvement in activities that diminish confidence in competence, impartiality, judgment or operational integrity.

e) Define the organization and management structure, as well as relationships between quality management, technical operations, and support services.

f) Specify the responsibility, authority and interrelationships of all personnel affecting quality of environmental tests. Documentation shall include a clear description of the lines of responsibility in the laboratory and shall be proportioned such that adequate supervision is ensured.

g) Provide adequate supervision of environmental testing staff, including trainees, by persons familiar with methods and procedures, purpose of each environmental test, and with the assessment of the environmental test results.

h) Have a technical manager with overall responsibility for the technical operations and provision of resources needed to ensure the required quality of laboratory operations. The technical director shall certify that personnel with appropriate educational and technical background perform all tests for which the laboratory is accredited, and shall meet the requirements specified in the accreditation process himself. This certification shall be documented (see Demonstration of Capability notebook).

i) Have a quality manager who is responsible for implementing the quality system and ensuring that it is followed at all times. The quality manager shall have direct access to the highest level of

management at which decisions are made on laboratory policy or resources. The quality manager may also be the technical director or deputy technical director. The quality manager shall:

1) Serve as the focal point for the QA/QC and be responsible for the oversight and/or review of quality control data.
2) Have functions independent from laboratory operations for which they have quality assurance oversight.
3) Be able to evaluate data objectively and perform assessments without outside influence.
4) Have documented training and/or experience in QA/QC procedures and be knowledgeable in the quality system as defined under NELAC.
5) Have a general knowledge of the analytical test methods for which data review is performed.
6) Arrange for or conduct internal audits as per 5.4.13 annually.
7) Notify laboratory management of deficiencies in the quality system and monitor corrective action.

j) Appoint deputies for key managerial personnel, including technical director and/or quality manager.

k) Participate in a proficiency test program for purposes of qualifying for and maintaining accreditation.

l) Establish and maintain data integrity procedures (see 5.11), including:

1) Data integrity training.
2) Signed data integrity documentation for all lab employees.
3) In-depth, periodic monitoring of data integrity.
4) Data integrity procedure documentation.

Responsibility

Lab Directors

References

None

Procedures

SOP Q-5
SOP Q-18
Employee Ethics and Data Integrity Agreement (Personnel file)

Quality Policy Manual 4.2 Quality System

Policy

Source Molecular Corporation maintains a formal quality system to ensure that testing conform to specified requirements. The quality system is designed to meet NELAC Standards

Quality System Requirements

4.2.1 Source Molecular Corporation has established implemented and maintains a quality system appropriate to its scope of activity. The laboratory documents its policies, systems, programs, procedures, and instructions to the extent necessary to assure the quality of the environmental test results. This documentation is communicated to, understood by, available to, and implemented by the appropriate personnel.

4.2.2 Source Molecular Corporation quality system policies and objectives are defined in a quality manual. The overall objectives are documented in a quality policy, and a statement issued under the authority of the Laboratory Directors and includes:

a) Management's commitment to good professional practice and quality of its tests. Policies and objectives for, and commitment to, accepted laboratory practices and quality of testing services is defined and documented.

b) Laboratory's standard of service.

c) Objectives of the quality system. These policies and objectives are documented in a quality manual.

d) Requirement that personnel familiarize themselves with the quality documentation and implement the policies and procedures in their work.

e) Management's commitment to compliance NELAC requirements.

4.2.3 The quality manual includes or makes reference to supporting procedures including Standard Operating Procedures, and outlines the structure of the documentation used. The quality manual and related quality documentation state the laboratory’s policies and operational procedures established in order to meet the requirements of NELAC standards.

4.2.4 The quality manual defines the roles and responsibilities of the technical and quality managers for ensuring compliance with NELAC.

Responsibility

Quality Manager – Dr. Troy Scott

References

None

Procedures

Quality Policy Manual — 4.3 Document Control

Policy

Source Molecular Corporation maintains a formal quality system for establishing and maintaining control.

Quality System Requirements

4.3.1 Source Molecular Corporation has procedures to control all documents that form part of its quality system, both internal and external documents (SOP Q-5).

4.3.2 Document Approval & Issue:

4.3.2.1 Documents issued as part of the quality system are reviewed and approved by authorized personnel. A master list identifying the current revision and distribution of documents in the quality system is used to ensure invalid and obsolete documents are not used. (SOP Q

4.3.2.2 The procedures ensures:

a) Authorized editions of documents are available, where necessary, for the effective functioning of the laboratory.

b) Documents are periodically reviewed and revised as necessary.

c) Invalid and obsolete documents are promptly removed from service, or assured against unintended use.

d) Obsolete documents retained are suitably marked.

4.3.2.3 Quality system documents generated are uniquely identified using the date of issue and/or revision identification, page numbering, the total number of pages or a mark indicating the end of the document, and the issuing authority.

4.3.3 Document Changes:

4.3.3.1 Changes are reviewed and approved by the same function that performed the original review. The designated person has access to pertinent background information.

4.3.3.2 Altered or new text is identified, where practical.

4.3.3.3 Hand amendments are clearly marked, initialed and dated. The revised document will be issued as soon as practical.

4.3.3.4 Computerized maintenance for documents is outlined in SOP Q-5.

Responsibility

Quality Manager – Dr. Troy Scott

References

SOP Q-5 Document control
SOP Q-17 Electronic Document Control for QC forms

Procedures

None

Quality Policy Manual 4.4 Review of Requests, Tenders and Contracts

Policy

Source Molecular Corporation ensures that all requirements received from the customer are fully understood and that current capability exists to meet all aspects of the customer's requirements prior to the acceptance of the contract.

Quality System Requirements

4.4.1 Policies and procedures for review leading to a contract for environmental testing ensure:

a) Requirements, including methods to be used, are adequately defined, documented and understood.

b) Source Molecular Corporation has the capability and resources to meet the requirements. Source Molecular Corporation will inform the client of any indicated potential conflict, deficiency, lack of appropriate accreditation status, or inability on the laboratory's part to complete the client's work.

c) Appropriate method is selected and can meet the client's requirements. Differences between request or tender and the contract are resolved before any work commences.

4.4.2 Records of reviews and pertinent discussions with clients are maintained.

4.4.3 Review includes any subcontracted work.

4.4.4 Clients are informed of deviation from contract.

4.4.5 Contracts amended after work starts have the same review as the original. Any change in accreditation status will be reported to the client.

Responsibility

Lab Directors

References

None

Procedures

SOP Q-20 (Review of requests, tenders, and contracts)

Quality Policy Manual 4.5 Subcontracting of Tests and Calibrations

Policy

Source Molecular Corporation does not employ subcontractors.

Quality System Requirements

None

Responsibility

Laboratory Directors

References

None

Procedures

None

Quality Policy Manual — 4.6 Purchasing Services and Supplies

Policy

Source Molecular Corporation ensures that products and services obtained for environmental tests conform to specified requirements.

Quality System Requirements

4.6.1 Source Molecular Corporation has policies and procedures for selection and purchasing of services and supplies that affect the quality of environmental tests.

4.6.2 Source Molecular Corporation ensures laboratory consumable materials are inspected or otherwise verified as complying with standard specifications or requirements defined in the methods for the environmental tests concerned, before use. Records of actions taken to check compliance are maintained.

4.6.3 Purchasing documents contain data describing the services and supplies ordered and are reviewed and approved for technical content prior to release.

4.6.4 Suppliers of critical consumables, supplies and services are evaluated, and records of the evaluations are maintained. A list of approved suppliers is maintained.

Responsibility

Accounts Receivable/Purchasing - Dr. Scott, Mr. Tamers

References

None

Procedures

SOP Q-4 Purchasing and Handling of Supplies

Quality Policy Manual — 4.7 Service to the Client

Policy

Source Molecular Corporation ensures that clients are assured of cooperation and service to the extent possible.

Quality System Requirements

Source Molecular Corporation affords clients cooperation to clarify request and monitor performance in relation to the work performed by the laboratory, to the extent that confidentiality of other clients is maintained.

Responsibility

Directors

References

None

Procedures

SOP Q-16 Ensuring Data Integrity

Quality Policy Manual — 4.8 Complaints

Policy

Source Molecular Corporation ensures that customer complaints are handled properly.

Quality System Requirements

Procedures exist for handling customer complaints. Records of complaints, Investigations, and corrective actions are maintained.

Responsibility

Directors

References

None

Procedures

Complaints are received, documented, and referred to Dr. Scott. Should errors be found, they are corrected and clients are notified within 48 hours. If no errors are found, clients are contacted within 48 hours 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.

Quality Policy Manual — 4.9 Control of Non-conforming Testing

Policy

Source Molecular Corporation ensures that testing that does not conform to specified requirements is prevented from unintended use. Control will provide for identification, documentation, evaluation, segregation, disposition, and notification of areas effected.

Quality System Requirements

4.9.1 Procedures exist and are implemented when work or the results of work do not conform to procedures or the requirements of the client.
The policies and procedures shall ensure:

a) Responsibility and authority for handling of nonconforming work are designated, and actions are defined and taken when nonconforming work is identified.(4.1.2.1)
b) Evaluation of the significance of the nonconformance.
c) Corrective action is taken immediately, together with any decision about the acceptability of the nonconforming work.
d) Where necessary, the client is notified.
e) Responsibility for authorizing the resumption of work is defined.

4.9.2 If nonconformance can recur, or there is doubt about compliance of the lab's operations with our own policies and procedures, Corrective Action procedure (SOP Q-1) is promptly followed.

Responsibility

Directors

References

None

Procedures

SOP Q-1 - Corrective Action
SOP Q-18 – Control of Nonconforming Testing

Quality Policy Manual 4.10 Corrective Action

Policy

Source Molecular Corporation utilizes documented procedures for corrective action to eliminate the causes of actual non-conformity.

Quality System Requirements

4.10.1 Policies and procedures are established for corrective action and are implemented by the Lab Director.

4.10.2 Cause Analysis (CA) is performed to investigate and determine the root cause.

4.10.3 Potential Corrective Action is identified, a determination is made to select and implement the appropriate corrective action that is likely to prevent recurrence, ensuring the Corrective Action is appropriate to the magnitude and risk of the problem. Any changes made as a result of the Corrective Action are documented and implemented.

4.10.4 Source Molecular Corporation monitors the Corrective Action to ensure that it is effective.

4.10.5 Where nonconformance or departures cast doubts on compliance with policies, procedures, or ISO/IEC 17025, the area of activity is audited per 4.13 as soon as possible.

4.10.6 Source Molecular Corporation establishes procedures to determine when departures from documented policies, procedures, and quality control have occurred, including:

4.10.6.1 Identifying individuals responsible for assessing each QC data type.

4.10.6.2 Identifying individuals responsible for initiating/recommending corrective actions.

4.10.6.3 Defining how the analyst shall treat a data set if the associated QC measurements are unacceptable.

4.10.6.4 Specifying how out-of-control situations and subsequent corrective actions are to be documented.

4.10.6.5 Specifying procedures for management to review corrective action reports.

4.10.7 To the extent possible, samples shall be reported only if all quality control measures are acceptable. If a quality control measure is found to be out of control and the data is to be reported, all samples associated with the failed quality control measure shall be reported with the appropriate laboratory defined data qualifiers.

Responsibility

Deviation from Standard Procedures: Dr. Scott
Corrective Action: Directors/Managers – Dr. Scott

Overview

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 director (T.M. Scott) or Sr. Research Scientist 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 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. Scott.

Procedures

Corrective Action (SOP Q-1)
Corrective Action for EPA Method 1623 (SOP Q-7)

Quality Policy Manual — 4.11 Preventive Action

Policy

Source Molecular Corporation utilizes documented procedures for preventive action to eliminate the causes of potential non-conformity.

Quality System Requirements

4.11.1 Improvements and potential nonconformances are identified. If preventive action is required, plans are developed, implemented and monitored to reduce the likelihood of occurrence and take advantage of opportunities for improvement.

4.11.2 Procedures include initiation of actions and controls to ensure they are effective.

Responsibility

Laboratory Manager

References

None

Procedures

QPM-001a sections 4.11.1 – 4.11.2

Quality Policy Manual 4.12 Control of Records

Policy

Source Molecular Corporation maintains accurate records to provide evidence that the quality system elements have been effectively implemented.

Quality System Requirements

4.12.1 General:

4.12.1.1 Procedures are established for identification, collection, indexing, access, filing, storage, maintenance and disposal of quality and technical records. These include reports from internal audits, management review and records of the corrective and preventative action process.

4.12.1.2 Records are legible and stored to be readily retrievable in suitable environments to prevent damage, deterioration or loss. Retention times of all records will be 5 years. Should the laboratory transfer ownership or go out of business, all records will be stored in a location suitable for all requirements in this section for a period of 5 years.

4.12.1.3 Records are held secure and in confidence.

4.12.1.4 Procedures to protect and back-up electronic records and prevent unauthorized access are in place.

4.12.1.5 The record keeping system allows historical reconstruction of all laboratory activities that produced the analytical data. Documentation allows the history of the sample to be readily understood.

4.12.1.5.1 Records include identity of personnel involved in sampling, sample receipt, preparation, and testing.

4.12.1.5.2 All information relating to laboratory facilities equipment, analytical test methods, and related laboratory activities, such as sample receipt, sample preparation, and data verification, are documented.

4.12.1.5.3 The record keeping system facilitates the retrieval of all working files and archived records for inspection and verification purposes.

4.12.1.5.4 All changes to records are initialed by responsible staff, and the reason for the initials is clearly indicated in the records, such as “sampled by”, “prepared by”, “or reviewed by”.

4.12.1.5.5 All generated data except those that are generated by automated data collection systems are recorded directly, promptly, and legibly in permanent ink.

4.12.1.5.6 Entries in records are not obliterated by methods such as erasures, overwritten files, or markings. Corrections to record-keeping errors (electronic and hard-copy) are made by one line marked through the error, and the individual making the correction initials and dates the correction.

4.12.2 Technical Records

4.12.2.1 Source Molecular Corporation retains records of original observations, derived data, and sufficient information to establish an audit trail, calibration, records, staff records, and a copy of each test report issued for a period of three years.

4.12.2.2 Observations, data and calculations are recorded at the time they are made and are identifiable to the specific task.

4.12.2.3 Mistakes are single-line crossed out, correct entry made, and signed or initialed by person making correction. Electronic records are handled to prevent change or loss of original data. When corrections are due to reasons other than transcription errors, the reason for the correction is documented.

4.12.2.4 Records management and storage

4.12.2.4.1 All records (including those pertaining to test equipment), certificates, and reports are safely stored, held secure, and in confidence to the client. NELAP-related records are available to the accrediting authority.

4.12.2.4.2 All records, including those specified in 4.12.2.5, are retained for a minimum of five years from the generation of the last entry in the records. The laboratory maintains all information necessary for the historical reconstruction of data. Records which are stored only on electronic media are supported by the hardware and software necessary for their retrieval.

4.12.2.4.3 Records that are stored or generated by computers have hard copy or write-protected backup copies.

4.12.2.4.4 Source Molecular Corporation has established a record management system for control of laboratory notebooks, instrument logbooks, standards logbooks, and records for data reduction, validation, storage, and reporting.

4.12.2.4.5 Access to archived information is documented with an access log. These records are protected against fire, theft, loss, environmental deterioration, vermin, and, in the case of electronic records, electronic and magnetic sources.

4.12.2.4.6 Source Molecular Corporation has a plan to ensure that records are maintained or transferred according to the clients' instructions in the event that the laboratory transfers ownership or goes out of business. In cases of bankruptcy, appropriate regulatory and state legal requirements concerning laboratory records will be followed.

4.12.2.5 Laboratory sample tracking

4.12.2.5.1 Sample handling: A record of all procedures to which a sample is subjected while in the possession of Source Molecular Corporation is maintained, including:

4.12.2.5.1.1 Sample preservation, including appropriateness of sample container and compliance with holding time requirement.

4.12.2.5.1.2 Sample identification, receipt, acceptance or rejection and log-in.

4.12.2.5.1.3 Sample storage and tracking, including shipping receipts, sample transmittal forms, and chain-of-custody form.

4.12.2.5.1.4 Documented procedures for the receipt and retention of samples, including all provisions necessary to protect the integrity of samples.

4.12.2.5.2 Laboratory support activities: the following are retained:

4.12.2.5.2.1 All original raw data, whether hard copy or electronic, for calibrations, samples, and quality control measures, including analysts' work sheets and data output records.

4.12.2.5.2.2 A written description or reference to the specific test method used which includes a description of the specific computational steps used to translate parametric observations into a reportable analytical value.

4.12.2.5.2.3 Copies of final reports.

4.12.2.5.2.4 Archived SOP's.

4.12.2.5.2.5 Correspondence relating to laboratory activities for a specific project.

4.12.2.5.2.6 All corrective action reports, audits, and audit responses.

4.12.2.5.2.7 Proficiency test results and raw data.

4.12.2.5.2.8 Results of data review, verification, and cross-checking procedures.

4.12.2.5.3 Analytical records: essential information associated with analysis, such as strip charts, tabular printouts, compute data files, analytical notebooks, and run logs, includes:

4.12.2.5.3.1 Laboratory sample ID code.

4.12.2.5.3.2 Date of analysis and time of analysis if the holding time is 72 hours or less, or when a time-critical step is included in the analysis, e.g. extractions, incubations.

4.12.2.5.3.3 Instrumentation, identification, and instrument operating conditions/parameters, or reference to such data.

4.12.2.5.3.4 Analysis type

4.12.2.5.3.5 All manual calculations, e.g. manual integrations.

4.12.2.5.3.6 Analyst's/operator's initials.

4.12.2.5.3.7 Sample preparation including cleanup, separation protocols, incubation periods or subculture, ID codes, volumes, weights, instrument printouts, meter readings, calculations, reagents.

4.12.2.5.3.8 Sample analysis.

4.12.2.5.3.9 Standard and reagent origin, receipt, preparation, and use.

4.12.2.5.3.10 Calibration criteria, frequency, and acceptance criteria.

4.12.2.5.3.11 Data and statistical calculations, review, confirmation, interpretation, assessment, and reporting conventions.

4.12.2.5.3.12 Quality control protocols and assessment.

4.12.2.5.3.13 Electronic data security, software documentation and verification, software and hardware audits, backups, and records of any changes to automated data entries.

4.12.2.5.3.14 Method performance criteria including expected quality control requirements.

4.12.2.5.4 Administrative records: the following is maintained:

4.12.2.5.4.1 Personnel qualifications, experience, and training records.

4.12.2.5.4.2 Records of demonstration of capability or each analyst.

4.12.2.5.4.3 A log of names, initials, and signatures for all individuals who are responsible for signing or initialing any laboratory record.

Responsibility

Office Manager

References

Procedures

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.

SOP Q-5 Document control
SOP Q-17 Electronic Document Control for QC forms

Quality Policy Manual — 4.13 Internal Audits

Policy

Source Molecular Corporation plans and implements internal audits to verify and assess the effectiveness of the company's Quality System.

Quality System Requirements

4.13.1 Source Molecular Corporation schedules and conducts annual internal audits of all activities that address all elements of NELAC Certification and the quality system using set procedures. Audits are planned and organized by the Quality Assurance manager and are carried out by trained and qualified personnel who are independent of activity being audited as resources permit. Personnel do not audit their own activities except when it can be demonstrated that an effective audit will be carried out.

4.13.2 When findings cast doubt on operations or validity of results, Source Molecular Corporation will take timely corrective action, and will notify clients in writing if investigations show results may have been affected, such as through identification of defective measuring or test equipment. Clients will be notified within 30 days.

4.13.3 The area of audits performed, findings and corrective actions are recorded. Laboratory management ensures that these actions are discharged within the agreed time frame as indicated in the quality manual or SOP's.

4.13.4 Follow-up activity is verified for implementation and effectiveness of corrective action and records are maintained.

Responsibility

Quality Manager

References

Procedures

Internal Audits as defined in SOP Q-1 (Corrective Action)

Quality Policy Manual — 4.14 Management Reviews

Policy

Source Molecular Corporation plans and implements management reviews to verify the suitability and the effectiveness of the quality System.

Quality System Requirements

4.14.1 Source Molecular Corporation schedules reviews of the quality system and environmental testing activities to insure their continued suitability and effectiveness, and to introduce changes or improvements. These reviews include:

4.14.1.1 Suitability of policies and procedures
4.14.1.2 Reports from mangers and supervisors
4.14.1.3 Outcome of recent internal audits
4.14.1.4 Corrective and preventive actions
4.14.1.5 Assessments by external bodies
4.14.1.6 Results of inter laboratory comparisons or proficiency tests
4.14.1.7 Changes in volume and type of work
4.14.1.8 Client feedback
4.14.1.9 Complaints
4.14.1.10 Other relevant factors

4.14.2 Records the findings and actions that arise from review. Management ensures that actions are carried out in a timely fashion. Source Molecular Corporation has a procedure for review by management, and maintains records of review findings and actions.

4.14.3 Source Molecular Corporation insures that a review is conducted with respect to any evidence of inappropriate actions or vulnerabilities related to data integrity. Discovery of potential issues is handled in a confidential manner until such time as a follow up evaluation, full investigation, or other appropriate actions have been completed and the issues clarified. All investigations that result in finding of inappropriate activity are documented and include any disciplinary actions involved, corrective actions taken, and all appropriate notifications of clients. All documentation of these investigation and actions taken are maintained for at least five years.

Responsibility

Quality Manager

References

Procedures

Review by management – SOP Q-1

Quality Policy Manual

4.15 Departure from Policies, Procedures, or Standards

Policy

Source Molecular Corporation management allows for exceptionally permitting departures from documented policies and procedures or from standard specifications.

Quality System Requirements

4.15.1 Departures from above stated policies, procedures, and specifications will be handled on a case-by-case basis.

Responsibility

Quality Manager

References

None.

Procedures

SOP Q-20
Handled on case basis

Quality Policy Manual

4.16 Protection of Confidentiality and Proprietary Rights

Policy

Source Molecular Corporation management is dedicated to protecting proprietary rights and confidentiality, including national security concerns.

Quality System Requirements

4.16.1 All empoyees of Source Molecular Corporation are verbally informed that they should protect company proprietary rights and client confidentiality.

4.16.2 All sensitive electronic documents are password protected.

Responsibility

Quality Manager

References

None.

Procedures

SOP Q-5

Quality Policy Manual

5.1 General Technical Requirements

Policy

Source Molecular Corporation ensures that various factors are considered to enable an effective delivery of service.

Quality System Requirements

5.1.1 The following factors are recognized by Source Molecular Corporation as contributing to the correctness and reliability of tests and/or calibrations:

5.1.1.1 Human factors

5.1.1.2 Accommodation and environmental conditions

5.1.1.3 Test and calibration methods and method validation

5.1.1.4 Equipment

5.1.1.5 Measurement traceability

5.1.1.6 The handling of test and calibration items

5.1.2 Source Molecular Corporation takes into account these factors while developing test and calibration methods and procedures, in the training and qualification of personel, and in the selection and calibration of the equipment used.

Responsibility

Laboratory Directors

References

Procedures

SOP Q-2 Environmental Monitoring
SOP Q-4 Handling of Supplies
SOP Q-15a Handling of Samples
SOP Q-15b Handling of PT Samples
SOP Q-16 Data Integrity
SOP Q-17 Document Control

Quality Policy Manual 5.2 Personnel

Policy

Source Molecular Corporation ensures the competence of all personnel who perform laboratory activities.

Quality System Requirements

5.2.1 Source Molecular Corporation ensures competence of all who operate equipment, perform tests, evaluate results, and sign test reports. While training is in progress, appropriate supervision is provided. Personnel performing specific tasks are qualified based on appropriate education, training, experience and/or demonstrated skills, as required. The laboratory has sufficient personnel with necessary education, training, technical knowledge and experience for their assigned functions. All personnel are responsible for complying with all quality assurance/quality control requirements that pertain to their organizational/technical function. Each technical staff member has a combination of experience and education to adequately demonstrate a specific knowledge of their particular function and a general knowledge of laboratory operations, test methods, quality assurance/quality control procedures and records management.

5.2.2 Source Molecular Corporation management formulates goals with respect to the education, training, and skills of the laboratory personnel. Policy and procedures are developed for identifying training needs and providing the training to meet these needs. This program takes into account present and anticipated task the laboratory may have.

5.2.3 Source Molecular Corporation uses personnel employed by or contracted to the laboratory. Where contractors or additional key personnel are used, supervision is provided to evaluate competence of work and adherence to the laboratory's quality system.

5.2.4 Job descriptions are maintained for managerial, technical and key support personnel involved in tests and calibrations.

5.2.5 Source Molecular Corporation authorizes specific personnel to perform particular types of tests, to issue test reports, and to give opinions and interpretations and to operate particular types of equipment. Records are maintained of the relevant authorizations, competence, educational and professional qualifications, training, skills and experience of all technical personnel, including contracted personnel. This information is readily available and includes the date on which authorization and/or competence is confirmed.

5.2.6 Laboratory management is responsible for:

5.2.6.1 Defining the minimal level of qualification, experience, and skills necessary for all positions in the laboratory. Basic laboratory skills shall be considered.

5.2.6.2 Ensuring that all technical laboratory staff have demonstrated capability in the acctivites for which they are responsible. Such demonstration is documented.

5.2.6.3 Ensuring that the training of eacch member of the technical staff is kept up-to-date by the following:

5.2.6.3.1 Evidence is on file that demonstrates that each employee has read, understood, and is using the latest version of the laboratory's in-house quality documentation that relates to his/her job responsibilities.

5.2.6.3.2 Training courses or workshops on specific equipment, analytical techniques, or laboratory procedures is documented.

5.2.6.3.3 Analyst training is considered up to date if an employee training file contains a certification that technical personnel have read, understood, and agreed to perform the most recent version of the test method and documentation of continued proficiency by at least one of the following once per year:

5.2.6.3.3.1 Acceptable performance of a blind sample (single blind to the analyst). NOTE: successful analysis of a blind perfromance sample on a similar test method using the same technology would only require documentation for one of the test methods. Acceptable limits of blind performance sample is determined prior to analysis.

5.2.6.3.3.2 An initial measurement system evaluation or another demonstration of capability.

5.2.6.3.3.3 At least four consecutive laboratory control samples with acceptable levels of precision and accuracy. The laboratory will determine the acceptable limits for precision and accuracy prior to analysis.

5.2.6.3.3.4 If 5.2.6.3.3.1 – 3 cannot be performed, analysis of authentic samples with results statistically indistinguishable form those obtained by another trained analyst.

5.2.6.3.4 Documenting all analytical and operational activities of the laboratory.

5.2.6.3.5 Supervising all personnel employed by the laboratory.

5.2.6.3.6 Ensuring that all sample acceptance criteria are verified and that samples are logged into the sample tracking system and properly labeled and stored.

5.2.6.3.7 Documenting the quality of all data reported by the laboratory.

5.2.7 Data integrity training is provided as a formal part of new employee orientation and is also provided on an annual basis for all current employees. Topics covered are documented in writing and provided to all trainees. Key topics covered during training include organizational mission and its relationship to the critical need for honesty and full disclosure in all analytical reporting, how and when to report data integrity issues, and record keeping. Training includes discussion regarding all data integrity procedures, data integrity training documentation, in-depth data monitoring and data integrity procedure documentation. Employees are informed that any infractions of the laboratory data integrity procedures will result in a detailed investigation that could lead to very serious consequences including immediate termination, debarment or civil/criminal prosecution. The initial data integrity training and the annual refresher training have a signature attendance sheet that demonstrates all staff have participated and understand their obligations related to data integrity. Senior managers acknowledge their support of these procedures by upholding the spirit and intent of Source Molecular Corporation's data integrity procedures, and effectively implementing the specific requirements of the procedures. Specific examples of breaches of ethical behavior are discussed, including improper data manipulations, adjustments of the instrument time clocks, and inappropriate changes in concentrations of standards. Data integrity training emphasizes the importance of proper written narration on the part of the analyst with respect to those cases where analytical

data may be useful, but are in one sense or another partially deficient. The data integrity procedures includes a written ethics agreement.

Responsibility

Laboratory Directors

References

Procedures

Training of Personnel (SOP Q-14)
Employee file

Quality Policy Manual

5.3 Accommodation and Environmental Conditions

Policy

Source Molecular Corporation ensures that proper accommodation and environmental conditions are provided for the laboratory facilities.

Quality System Requirements

5.3.1 Source Molecular Corporation ensures those laboratory facility conditions such as energy sources, lighting and environmental conditions are adequate to facilitate the correct performance of the test. The technical requirements for accommodation and environmental conditions that can affect the results of tests and calibration are documented and are met before testing begins; this includes when testing occurs at sites other than the permanent facility.

5.3.2 Source Molecular Corporation monitors, controls and records environmental conditions where necessary to maintain quality of testing and calibration as required by the relevant specifications, methods and procedures or where they influence the quality of the results. Electrical supply, temperature, sound and vibration levels, biological sterility, dust, electromagnetic disturbances, radiation, and humidity as appropriate to the technical activities, are taken into consideration. Tests and calibration will be stopped when environmental conditions jeopardize the results of the tests. In instances where monitoring or control of any of the above mentioned items is specified in a test method or by regulation, Source Molecular Corporation meets and documents adherence to the laboratory facility requirements.

5.3.3 There is effective separation between work areas that are incompatible to prevent cross contamination.

5.3.4 Access to areas affecting the quality of the tests is controlled and such control is appropriate to the particular circumstances.

5.3.5 Good housekeeping measures are taken with special procedures as needed.

5.3.6 Work spaces are available to ensure an unencumbered work area. Work areas include:

5.3.6.1 Access and entryways to the laboratory.

5.3.6.2 Sample receipt area.

5.3.6.3 Sample storage area.

5.3.6.4 Chemical and waste storage area.

5.3.6.5 Data handling and storage areas.

5.3.7 Floors and work surfaces are non-absorbent and easy to clean and disinfect. Work surfaces are adequately sealed. Source Molecular Corporation provides sufficient storage space, and the laboratory is clean and free from dust accumulation. Plants, food, and drink are prohibited from the laboratory work area.

Responsibility

President

References

Procedures

Environmental Monitoring (SOP Q-2)
Dishwashing (SOP Q-6)
Autoclaving (SOP Q-13)

Policy

Source Molecular Corporation ensures that the test methods are validated and implemented accordingly.

Quality System Requirements

5.4.1 General: Source Molecular Corporation uses appropriate methods and procedures within its scope. Methods and procedures include handling, transport, storage and preparation of samples, and where appropriate, an estimation of the measurement uncertainty as well as statistical techniques for analysis of test data. Instructions on the use and operation of all relevant equipment, and on the handling and preparation of samples are provided where the absence of such instructions could jeopardize the test results. Instructions, standards, manuals and reference data relevant to the work of the laboratory is kept up to date and made readily available to personnel as necessary. Deviation from test and/or calibration methods are implemented when such deviations are documented, technically justified, authorized and accepted by the client.

5.4.1.1 Standard Operating Procedures (SOP's): Source Molecular Corporation maintains SOP's that accurately reflect all phases of current laboratory activities such as assessing data integrity, corrective actions, handling customer complaints, and all test methods.

5.4.1.1.1 These documents may include equipment manuals provided by the manufacturer or internally written documents with adequate detail to allow someone similarly qualified, other than the analyst, to reproduce the procedures used to generate the test result.

5.4.1.1.2 Test methods may include copies of published methods, provided any changes or selected options in the methods are documented and included in the methods manual.

5.4.1.1.3 Copies of all SOP's are accessible to all personnel.

5.4.1.1.4 SOP's are organized.

5.4.1.1.5 Each SOP clearly indicate the effective data of the document, the revision number, and the signatures of the approving authority.

5.4.1.1.6 The documents specified in 1 and 2 that contain sufficient information to perform tests do not need to be supplemented or rewritten as internal procedures, if the documents are written in a way that they can be used as written. Any changes, including the use of a selected option is documented and included in Source Molecular Corporation's methods manual.

5.4.1.2 Laboratory Method Manuals:

5.4.1.2.1 Source Molecular Corporation has and maintains an in-house methods manual for each accredited analyte or test method.

5.4.1.2.2 This manual may consist of copies of published or referenced test methods or SOP's that have been written by the laboratory. In cases where modifications to the published method have been made by the laboratory or where the referenced test method is ambiguous or provides insufficient detail, these changes or clarifications are clearly described. Each test method includes or references where applicable:

5.4.1.2.2.1 Identificaiton of the test method.

5.4.1.2.2.2 Applicable matrix or matrices.

5.4.1.2.2.3 Detection limit.

5.4.1.2.2.4 Scope and application, including components to be analyzed.

5.4.1.2.2.5 Summary of the test method.

5.4.1.2.2.6 Definitions.

5.4.1.2.2.7 Interferences.

5.4.1.2.2.8 Safety.

5.4.1.2.2.9 Equipment and supplies.

5.4.1.2.2.10 Reagents and standards.

5.4.1.2.2.11 Sample collection, preservation, shipment and storage.

5.4.1.2.2.12 Quality control.

5.4.1.2.2.13 Calibration and standardization.

5.4.1.2.2.14 Procedure.

5.4.1.2.2.15 Data analysis and calculations.

5.4.1.2.2.16 Method performance.

5.4.1.2.2.17 Pollution prevention.

5.4.1.2.2.18 Data assessment and acceptance criteria for quality control measures.

5.4.1.2.2.19 Corrective actions for out-of-control data.

5.4.1.2.2.20 Contingencies for handling out-of-control or unacceptable data.

5.4.1.2.2.21 Waste management.

5.4.1.2.2.22 References.

5.4.1.2.2.23 Any tables, diagrams, flowcharts, and validation data.

5.4.2 Selection of Methods: Source Molecular Corporation uses methods for environmental testing which meet the needs of the client and which are appropriate for the environmental tests it undertakes.

5.4.2.1 Sources of Methods:

5.4.2.1.1 Methods published in international, regional or national standards are preferably used. Source Molecular Corporation ensures the use of latest valid edition of standards, unless it is not appropriate or possible to do so. When necessary, standards are supplemented with additional details to ensure consistent application

5.4.2.1.2 When the use of specific methods for a sample analysis is mandated or requested, only those methods are used.

5.4.2.1.3 When the client does not specify the method to be used or where methods are employed that are not required, the methods are fully documented and validated and available to the client and other recipients of the relevant reports. Source Molecular Corporation shall select appropriate published methods when client has not specified a method. Laboratory developed methods and methods adopted by the laboratory are used if appropriate and are validated. Client is informed of method chosen.

5.4.2.1.4 Client is informed if the standard proposed by the client is considered to be inappropriate or out of date.

5.4.2.2 Source Molecular Corporation confirms that it can perform standard methods before introducing the tests. If standard method changes, confirmation is repeated.

5.4.2.2.1 Prior to acceptance and institution of any method, satisfactory demonstration of method capability is demonstrated using clean quality matrix sample, e.g. drinking water, solids, biological tissue or air. For analytes which do not lend themselves to spiking, the demonstration of capability may be performed using quality control samples.

5.4.2.2.2 Thereafter, continuing demonstration of method performance, as per the quality control requirements, such as laboratory control samples, is required.

5.4.2.2.3 In cases where the laboratory analyzes samples using a method that has been in use by the laboratory before July 1999, and there have been no significant changes in instrument type, personnel or method, the continuing demonstration of method performance and the analyst's documentation of continued proficiency is acceptable. The laboratory shall have records on fill to demonstrate that a demonstration of capability is not required.

5.4.2.2.4 In all cases, the appropriate forms such as the Certification Statement are completed and retained by the laboratory and available upon request. All associated supporting data necessary to reproduce the analytical results summarized in the Certification Statement are retained by the laboratory.

5.4.2.2.5 A demonstration of capability will be completed each time there is a change in instrument type, personnel, or method.

5.4.2.2.6 Source Molecular Corporation does not use specialized work cells.

5.4.3 Laboratory-developed Methods

- The introduction of methods developed by the lab is a planned activity and development is assigned to qualified personnel equipped with adequate resources.
- Plans shall be updated as development proceeds and communication among personnel involved will be ensured.

5.4.4 Non-standard Methods

- Non-standard methods are subject to agreement with the client, and include specification of client's requirements and purpose of the test. Method developed shall be validated before use.

5.4.5 Validation of Methods

5.4.5.1 Validation is confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.

5.4.5.2 Non-standard and laboratory-designed/developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods are validated to confirm that they are fit for the intended use. The validation is as extensive as is necessary to meet the needs of the given application or field of application. The laboratory records the results obtained, the procedure used for the validation, and a statement as to whether the method is fit for the intended use. The minimum requirements are the initial test method evaluation requirements.

5.4.5.3 The range and accuracy of the values obtainable from validated methods shall be relevant to the client's needs.

5.4.6 Estimation of uncertainty of measurement

5.4.6.1 Source Molecular Corporation has and applies procedures to estimate the uncertainty of measurement. When the nature of the test method precludes rigorous, metrologically and statistically valid calculation of uncertainty of measurement, the laboratory shall attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data. In cases where a well-recognized test method specifies limits to the values of the major sources of uncertainty of measurement and specifies the form of presentation of calculated results, the laboratory will simply follow the test method and reporting instructions.

5.4.6.2 All uncertainty components which are of importance in a given situation shall be taken into account using appropriate methods of analysis when estimating uncertainty of measurement.

5.4.7 Control of data:

5.4.7.1 Calculations and data transfers are subject to appropriate checks in a systematic manner

5.4.7.1.1 Source Molecular Corporation as established SOP’s to ensure that the reported data are free from transcription and calculation errors.

5.4.7.1.2 Source Molecular Corporation as established SOP’s to ensure that all quality control measures are reviewed and evaluated before data are reported.

5.4.7.1.3 Source Molecular Corporation as established SOP’s addressing manual calculations including integrations.

5.4.7.2 When computers, automated equipment, or microprocessors are used for the acquisition, processing, recording, storage, or retrieval of environmental test data, the laboratory ensures that:

5.4.7.2.1 Computer software developed by the user is documented in sufficient detail and is suitably validated as being adequate for use.

5.4.7.2.2 Procedures are established and implemented for protecting the data, including integrity and confidentiality of data entry and collection, data storage, data transmission, and data processing.

5.4.7.2.3 Computers and automated equipment are maintained to ensure proper functioning and are provided with the environmental and operating conditions necessary to maintain the integrity of environmental test data.

5.4.7.2.4 Appropriate procedures are implemented for maintenance of security of data including the prevention of unauthorized access to, and the unauthorized amendment of, computer records.

5.4.7.2.5 Commercial off-the-shelf software in general use within their designed application range is considered to be sufficiently validated. Laboratory software configuration or modifications are validated as above.

5.4.8 Method Evaluation:

5.4.8.1 Source Molecular Corporation demonstrates proficiency with a test method prior to first use. This is achieved by comparison to a method already approved for use in the laboratory, or by analyzing a minimum of ten spiked samples whose quality system matrix is representative of those normally submitted to the laboratory, or by analyzing and passing one proficiency test series provided by an approved proficiency sample provider. The laboratory maintains this documentation as long as the method is in use and for at least 5 years past the date of last use.

5.4.8.2 Source Molecular Corporation participates in the Proficiency Test programs identified by NELAP. The results of these analyses are used to evaluate the ability of the laboratory to produce acceptable data.

Responsibility

Laboratory Director

References

Procedures

Balance Calibration (SOP Q-9)
Microscope Calibration (SOP Q-10)
PH meter calibration (SOP Q-3)
Thermometer Calibration (SOP Q-8)

See Specific Testing Method Manuals

Policy

Source Molecular Corporation ensures the proper equipment is available and is suitable for the delivery of intended service.

Quality System Requirements

5.5.1 Lab is furnished with all items of equipment required for correct performance of testing. Equipment outside permanent control shall be controlled to meet all standards

5.5.2 Equipment and software meet the accuracy necessary for the testing and comply with specifications relevant to environmental tests concerned. Equipment shall be calibrated or checked to establish that it meets the specification requirements and complies with relevant standards before being put into service.

5.5.2.1 Support Equipment:

5.5.2.1.1 All support equipment is maintained in proper working order. The records of all repair and maintenance activities including service calls are kept on file.

5.5.2.1.2 All support equipment is calibrated or verified annually, using NIST traceable references when available, over the entire range of use. The results of such calibration or verification shall be within the specifications required of the application for which this equipment is used or:

5.5.2.1.2.1 The equipment shall be removed from service until repaired or

5.5.2.1.2.2 The laboratory shall maintain records of established correction factors to correct all measurements.

5.5.2.1.3 Raw data records are retained to document equipment performance.

5.5.2.1.4 Prior to use on each working day, balances, ovens, refrigerators, freezers, and water baths are checked in the expected use range, with NIST traceable references where commercially available. The acceptability for use or continued use shall be according to the needs of the analysis or application for which the equipment is being used.

5.5.2.2 Instrument Calibration:

5.5.2.2.1 Initial instrument calibration:

5.5.2.2.1.1 The details of the initial instrument calibration procedures including calculations, integrations, acceptance criteria and associated statistics are included or referenced in the test method SOP. When initial instrument calibration procedures are referenced in the test method, then the referenced material will be retained by the laboratory and be available for review.

5.5.2.2.1.2 Sufficient raw data records will be retained to permit reconstruction of the initial instrument calibration, e.g., calibration date, test method, instrument, analysis date, each analyte name, analyst's initials, concentration and response, calibration curve or response factor, or unique equation or coefficient used to reduce instrument responses to concentration.

5.5.2.2.1.3 Sample results are quantitated from the initial instrument calibration and may not be quantitated from any continuing instrument calibration verification unless otherwise required by regulation, method, or program.

5.5.2.2.1.4 All initial instrument calibrations will be verified with a standard obtained from a second manufacturer or lot if the lot can be demonstrated from the manufacturer as prepared independently from other lots. Traceability shall be to a national standard, when commercially available.

5.5.2.2.1.5 Criteria for the acceptance of an initial instrument calibration will be established, e.g., correlation coefficient or relative percent difference. The criteria used will be appropriate to the calibration technique employed.

5.5.2.2.1.6 The lowest calibration standard shall be the lowest concentration for which quantitative data are to be reported. Any data reported below the lower limit of quantitation will be considered to have an increased quantitative uncertainty and shall be reported using defined qualifiers or flags or explained in the case narrative.

5.5.2.2.1.7 The highest calibration standard shall be the highest concentration for which quantitative data are to be reported. Any data reported above this highest standard will be considered to have an increased quantitative uncertainty and shall be reported using defined qualifiers or explained in the case narrative.

5.5.2.2.1.8 Measured concentrations outside the working range shall be reported as having less certainty and shall be reported using defined qualifiers or flags or explained in the case narrative. The lowest calibration standard must be above the limit of detection. Noted exception: the following shall occur for instrument technology with validated techniques from manufacturers or methods employing standardization with a zero point and a single point calibration standard:

5.5.2.2.1.8.1 Prior to the analysis of samples the zero point and single point calibration will be analyzed and the linear range of the instrument will be established by analyzing a series of standards, one of which must be at the lowest quantitation level. Sample results within the established linear range will not require data qualifier flags.

5.5.2.2.1.8.2 Zero point and single-point calibration standard must be analyzed with each analytical batch.

5.5.2.2.1.8.3 A standard corresponding to the limit of quantitation must be analyzed with each analytical batch and must meet established acceptance criteria.

5.5.2.2.1.8.4 The linearity is verified at a frequency established by the method and/or the manufacturer.

5.5.2.2.1.9 If the initial instrument calibration results are outside established acceptance criteria, corrective actions will be performed and all associated samples will be reanalyzed. If reanalysis of the samples is not possible, data associated with an unacceptable initial instrument calibration shall be reported with appropriate data qualifiers.

5.5.2.2.1.10 If a reference or mandated method does not specify the number of calibration standards, the minimum number is two, one of which must be at the limit of quantitation, not including blanks or a zero standard with the noted exception of instrument technology for which it has been established by methodologies and preocedures that a zero and a single point standard are appropriate for calibrations. The laboratory will have a SOP for determining the number of points for establishing the initial instrument calibration.

5.5.3 Equipment is operated by authorized personnel. Up to date instructions for the equipment use and maintenance are readily available. All equipment is properly maintained, inspected, and cleaned. Maintenance procedures are documented.

5.5.4 Equipment and software are uniquely identified.

5.5.5 Source Molecular Corporation maintains records for each major item of equipment significant to the tests performed, including:

5.5.5.1 Identity of item of equipment

5.5.5.2 Manufacturer’s name, type, identification and serial number or other unique identification.

5.5.5.3 Checks that equipment complies with the specification.

5.5.5.4 Current location, where appropriate.

5.5.5.5 Manufacturer’s instructions, if available, or reference to their location.

5.5.5.6 Dates, results and copies of reports and certificates of calibration, adjustments, acceptance criteria, and due date of next calibration

5.5.5.7 Maintenance plan, where appropriate, and maintenance carried out to date’ documentation on all routine and non-routine maintenance activities and reference material verifications.

5.5.5.8 Damage, malfunction, modification or repair to equipment.

5.5.5.9 Date received and date placed in service, if available.

5.5.5.10 Condition when received, if available.

5.5.6 Procedures are established for safe handling, transport, storage, use and planned maintenance of the measuring equipment.

5.5.7 Overloaded or mishandled equipment that gives suspect results are taken out of service, isolated and labeled accordingly until it has been repaired and calibrated. Effect of the defect or departure from previous limits on previous testing and/or calibration are examined and "Control of nonconforming work" procedures are initiated.

5.5.8 Equipment shall be labeled, coded or otherwise identified to indicate status of calibration, including date calibrated, and date or expiration criteria when recalibration is due.

5.5.9 If equipment goes outside the control of the lab, it shall be proven that the function and calibration status are satisfactory before being returned to service.

5.5.10 When an initial instrument calibration is not performed on the day of analysis, the validity of the initial calibration is verified prior to sample analyses by continuing instrument calibration verification with each analytical batch. The following items are essential elements of continuing instrument verification:

5.5.10.1 The details of the continuing instrument calibration procedure, calculations, and associated statistics are included or referenced in the test method SOP.

5.5.10.2 Instrument calibration verification is performed:

5.5.10.2.1 At the beginning and end of each analytical batch (except, if an internal standard is used, only one verification is performed at the beginning of the analytical batch).

5.5.10.2.2 Whenever it is expected that the analytical system may be out of calibration or might not meet the verification acceptance criteria.

5.5.10.2.3 If the time period for calibration or the most previous calibration verification has expired.

5.5.10.2.4 For analytical systems that contain a calibration verification requirement.

5.5.10.3 Sufficient raw data records are retained to permit reconstruction of the continuing instrument calibration verification, e.g. test method, instrument, analysis date, each analyte name, concentration and response, calibration curve or response factor, or unique equations or coefficients used to convert instrument responses into concentrations. Continuing calibration verification records explicitly connect the continuing verification data to the initial instrument calibration.

5.5.10.4 Criteria for the acceptance of a continuing instrument calibration verification is established. If the continuing instrument calibration verification results obtained are outside established acceptance criteria, corrective actions are performed. If routine corrective action procedures fail to produce a second consecutive, immediate, calibration verification within acceptance criteria, then either the laboratory will demonstrate acceptable performance after corrective action with two consecutive calibration verifications, or a new initial instrument calibration will be performed. If the laboratory has not verified calibration, sample analyses will not occur until the analytical system is calibrated or calibration verified. If samples are analyzed using a system on which the calibration has not yet been verified, the results will be flagged. Data associated with an unacceptable calibration verification may be fully useable under the following special conditions:

5.5.10.4.1 When the acceptance criteria for the continuing calibration verification are exceeded high, i.e. high bias, and there are associated samples that are non-detects, then those non-detects may be reported. Otherwise the samples affected by the unacceptable calibration verification shall be reanalyzed after a new calibration curve has been established, evaluated, and accepted.

5.5.10.4.2 When the acceptance criteria for the continuing calibration verification are exceeded low, i.e. low bias, those sample results may be reported if they exceed a maximum regulatory limit/decision level. Otherwise the samples affected by the unacceptable verification shall be reanalyzed after a new calibration curve has been established, evaluated, and accepted.

5.5.11 Where calibrations give rise to a set of correction factors, Source Molecular Corporation provides procedures to ensure that copies are updated correctly. (4.5)

5.5.12 Equipment and software is safeguarded from adjustments that would invalidate the testing and calibration results. (4.12)

5.5.13 Temperature Measuring Devices such as liquid-in-glass thermometers, thermocouples, and platinum resistance thermometers used in incubators, autoclaves and other equipment is the appropriate quality to meet specifications in the test method. The graduation of the temperature measuring devices is appropriate for the required accuracy of measurement, and they are calibrated to national or international standards for temperature, at least annually. (5.6.2.2.2)

5.5.14 Autoclaves:

5.5.14.1 The performance of each autoclave is initially evaluated by establishing its functional properties and performance, for example heat distribution characteristics with respect to typical uses. Autoclaves meet specified temperature tolerances. Pressure cookers are not used for sterilization of growth media.

5.5.14.2 Demonstration of sterilization temperature is provided by use of a maximum registering thermometer with every cycle. Appropriate biological indicators are used once per month to determine effective sterilization. Temperature sensitive tape is used with the contents of each autoclave run to indicate that the autoclave contents have been processed.

5.5.14.3 Records of autoclave operations are maintained for every cycle. Records include: date, contents, maximum temperature reached, pressure, time in sterilization mode, total run time (may be recorded as time in and time out) and analyst's initials.

5.5.14.4 Autoclave maintenance, either internally or by service contract, is performed annually and includes a pressure check and calibration of temperature device. Records of the maintenance is maintained in equipment logs.

5.5.14.5 The autoclave mechanical timing device is checked quarterly against a stopwatch and the actual time elapsed documented.

Responsibility

Laboratory Directors

Calibration and Maintenance of Laboratory equipment

pH meters - All pH meters are calibrated within + 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.

Balances - All balances are calibrated monthly using ASTM (NIST traceable) type weights at three (3) different points. In addition, professional calibration of all balances occurs at least once annually.

Incubators – All incubators are maintained at their desired temperature + 0.5 ^{o}C or +0.2 ^{o}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 T. M. Scott. Documentation must be provided as to steps taken to correct problems as they arise. The problem log is located in the QC notebook.

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.

Sterilization procedures - All items are sterilized in the autoclave at 121^{o}C for a minimum of 15 minutes. Biohazardous wastes are sterilized for a minimum of 30 minutes.

Refrigerators - All refrigerators/freezers are monitored to maintain a temperature of 1-8 ^{o}C by a bulb thermometer immersed in glycerol.

Procedures

pH Meter Calibration (SOP Q-3)
Thermometer Calibration (SOP Q-8)
Balance Calibration (SOP Q-9)
Microscope Calibration (SOP Q-10)
Equipment List and Maintenance Log

Quality Policy Manual — 5.6 Measurement Traceability

Policy

Source Molecular Corporation ensures that all equipment used for tests and/or calibration are calibrated and are traceable to National and/or International standards.

Quality System Requirements

5.6.1 General

5.6.1.1 All equipment used for testing, including equipment for subsidiary measurements and having significant effect on the accuracy or validity of the results of the test are calibrated before being put into service and on a continuing basis. This includes balances, thermometers, and control standards. Such a program shall include a system for selecting, using, calibrating, checking, controlling and maintaining measurement standards, reference materials used as measurement standards, and measuring and test equipment used to perform environmental tests.

5.6.2 Testing Laboratories:

5.6.2.1 Source Molecular Corporation ensures that the equipment used can provide the uncertainty of measurement needed.

5.6.2.1.1 The overall program of calibration and/or verification and validation of equipment is designed and operated so as to ensure that measurements made by the laboratory are traceable to national standards of measurement.

5.6.2.2 Where traceability of measurements to SI units is not possible or not relevant, the same requirements for traceability to, for example, certified reference materials, agreed methods and/or consensus standards, are in effect. The laboratory provides satisfactory evidence of correlation of results.

5.6.3 Reference Standards and Reference Materials:

5.6.3.1 Reference standards: the laboratory has a program and procedure for the calibration of its reference standards. Reference standards are calibrated by a body that can provide traceability as described in 5.6.2.1. Such reference standards of measurement held by the laboratory (such as class S or equivalent weights or traceable thermometers) are used for calibration only and for no other purpose, unless it can be shown that their performance as reference standards would not be invalidated. Reference standards are calibrated before and after any adjustment. Where commercially available, this traceability is to a national standard of measurement.

5.6.3.2 Reference materials: Reference materials are, where commercially available, traceable to SI units of measurement, or to certified reference materials. Where possible, traceability is to national or international standards of measurement, or to national or international standard reference materials. Internal reference materials are checked as far as is technically and economically practicable.

5.6.3.3 Intermediate checks: Checks needed to maintain confidence in the status of reference, primary, transfer, or working standards and reference materials are carried out according to defined procedures and schedules.

5.6.3.4 Transport and storage: the laboratory has procedures for safe handling, transport, storage, and use of reference standards and reference materials in order to prevent contamination or deterioration and in order to protect their integrity.

5.6.4 Source Molecular Corporation, has documented procedures for the purchase, reception, and storage of consumable materials used for the technical operations of the laboratory.

5.6.4.1 The laboratory retains records for all standards, reagents, reference materials, and media including the manufacturer/vendor, the manufacturer's Certificate of Analysis or purity, if supplied, the date of receipt, recommended storage conditions, and an expiration date after which the material hall not be used unless its reliability is verified by the laboratory.

5.6.4.2 Original containers, such as provided by the manufacturer or vendor, are labeled with an expiration date.

5.6.4.3 Records are maintained on standard and reference material preparation. These records indicate traceability to purchased stocks or neat compounds, reference to the method of preparation, date of preparation, expiration date, and preparer's initials.

5.6.4.4 All containers of prepared, standards, and reference materials will bear a unique identifier and expiration date and be linked to the documentation requirements in 5.6.4.3.

5.6.4.5 Procedures are in place to ensure prepared reagents meet the requirements of the test method. The source of reagents complies with 5.9.2.1.6 and D.1.4.2.

5.6.4.6 All containers of prepared reagents bear a preparation date. An expiration date is defined on the container or documented elsewhere as indicated in the laboratory's quality manual or SOP.

Responsibility

Laboratory Directors

References

Procedures

pH Meter Calibration (SOP Q-3)
Thermometer calibration (SOP Q-8)
Balance Calibration (SOP Q-9)
Microscope Calibration (SOP Q-10)
Reference Standards and Materials (SOP Q-21)
Calibration Logs located at each instrument and filed in calibration log notebook

Quality Policy Manual 5.7 Sampling

Policy

Source Molecular Corporation performs sampling under the current scope through set sampling plans and procedures.

Quality System Requirements

5.7.1 Source Molecular Corporation has a sampling plan and procedures for sampling when it carries out sampling of substances, materials, or products for subsequent environmental testing. The sampling plan as well as the sampling procedure is made available at the location where the sampling is undertaken. Sampling plans are, whenever reasonable, based on appropriate statistical methods. The sampling process addresses the factors to be controlled to ensure the validity of the environmental test results. Where sampling (as in obtaining sample aliquots from a submitted sample) is carried out as part of the test method, the laboratory uses documented procedures and appropriate techniques to obtain representative subsamples.

5.7.2 Where the client requires deviations, additions, or exclusions from the documented sampling procedure, these are recorded in detail with the appropriate sampling data and are included in all documents containing environmental test and/or calibration results, and are communicated to the appropriate personnel.

5.7.3 The laboratory has procedures for recording relevant data and operations relating to sampling that forms part of the environmental testing that is undertaken. These records include the sampling procedure used, the identification of the sampler, environmental conditions (if relevant) and diagrams or other equivalent means to identify the sampling location as necessary and, if appropriate, the statistics the sampling procedures are based upon.

Responsibility

Laboratory Directors

References

Procedures

Field Quality Control Requirements (SOP FS-1)
Field Temperature (SOP FS-2)
Field pH (SOP FS-3)
Wastewater and Sludge Sampling (SOP FS-4)
Surface Water Sampling (SOP FS-5)

Policy

Source Molecular Corporation ensures that samples are controlled through handling, storage, packaging, preservation, and delivery in such a manner that product integrity is maintained.

Quality System Requirements

5.8.1 Source Molecular Corporation has procedures for transportation, receipt, handling, protection, storage, retention and/or disposal of samples, including all provisions necessary to protect sample integrity and the interests of the laboratory and the client.

5.8.2 Samples are identified and identity is retained throughout life of the sample in the lab. Samples are identified to ensure they cannot be confused physically or when referred to in records and other documents. Sample identification accommodates sub-division of groups and transfer within and from the laboratory.

5.8.2.1 The laboratory has a documented system for uniquely identifying the samples to be tested, to ensure that there can be no confusion regarding the identity of such samples at any time. This system includes identification for all samples, subsamples, and subsequent extracts and/or digestates. The laboratory assigns a unique identification code to each sample container received in the laboratory.

5.8.2.2 The laboratory code maintains an unequivocal link with the unique field ID code assigned each container.

5.8.2.3 The laboratory ID code is placed on the sample container as a durable label.

5.8.2.4 The laboratory ID code is entered in to the laboratory records and is the link that associates the sample with related laboratory activities such as sample preparation.

5.8.2.5 The laboratory ID code may be the same as the field ID code.

5.8.3 Upon receipt of a sample, the condition, including abnormalities or departures from normal or specified conditions are recorded. When suitability is in doubt, or when a sample does not conform to the description provided, or the environmental test required is not specified in sufficient detail, the client is consulted for further instructions before proceeding and discussions are recorded.

5.8.3.1 Sample receipt protocols:

5.8.3.1.1 All items specified in 5.8.3.2 shall be checked.

5.8.3.1.1.1 All samples which require thermal preservation shall be considered acceptable if the arrival temperature is either within 2 C of the required temperature or the method specified range. For samples with a specified temperature of 4 C, samples with a temperature ranging from just above the freezing temperature of water to 6 C shall be acceptable. Samples that are hand delivered to the laboratory on the same day that they are collected may not meet these criteria. In these cases, the samples shall be considered acceptable if there is evidence that the chilling process has begun such as arrival on ice.

5.8.3.1.1.2	The laboratory has implemented procedures for checking chemical preservation using readily available techniques, such as pH or chlorine, prior to or during sample preparation or analysis.
5.8.3.1.1.3	Microbiological samples from chlorinated water systems do not require an additional chlorine residual check in the laboratory if the following conditions are met:
5.8.3.1.1.3.1	Sufficient sodium thiosulfate is added to each container to neutralize at minimum 5 mg/L of chlorine for drinking water and 15 mg/L of chlorine for wastewater samples.
5.8.3.1.1.3.2	One container from each batch of laboratory prepared containers or lot of purchased ready-to-use containers is checked to ensure efficacy of the sodium thiosulfate to 5 mg/L of chlorine or 15 mg/L of chlorine as appropriate and the check is documented.
5.8.3.1.1.3.3	Chlorine residual is checked in the field and actual concentration is documented with sample submission.
5.8.3.1.2	The results of all checks is recorded.
5.8.3.1.3	If the sample does not meet the sample receipt acceptance criteria listed in this standard, the laboratory shall either:
5.8.3.1.3.1	Retain correspondence and/or records of conversations concerning the final disposition of rejected samples, or
5.8.3.1.3.2	Fully document any decision to proceed with the analysis of samples not meeting acceptance criteria.
5.8.3.1.3.2.1	The condition of these samples shall, at a minimum, be noted on the chain of custody or transmittal form and laboratory receipt documents.
5.8.3.1.3.2.2	The analysis data shall be appropriately “qualified” on the final report.
5.8.3.1.4	Source Molecular Corporation utilizes a permanent chronological record to document receipt of all sample containers.
5.8.3.1.4.1	This sample receipt log records the following:
5.8.3.1.4.1.1	Client/project name.
5.8.3.1.4.1.2	Date and time of laboratory receipt.
5.8.3.1.4.1.3	Unique laboratory ID code (5.8.2).
5.8.3.1.4.1.4	Signature or initials of the person making the entries.
5.8.3.1.4.2	During the log-in process, the following information is unequivocally linked to the log record or included as part of the log. If such information is recorded/documented elsewhere, the records are part of the laboratory’s permanent records, easily retrievable upon request and readily available to individuals who will process the sample.
5.8.3.1.4.2.1	The field ID code which identifies each container is linked to the laboratory ID code in the sample receipt log.
5.8.3.1.4.2.2	The date and time of sample collection is linked to the sample container and to the date and time of receipt in the laboratory.
5.8.3.1.4.2.3	The requested analyses, including applicable approved test method numbers, are linked to the laboratory ID code.

5.8.3.1.4.2.4 Any comments resulting from inspection for sample rejection are linked to the laboratory code.

5.8.3.1.5 All documentation, such as memos or transmittal forms, that is transmitted to the laboratory by the sample transmitter are retained.

5.8.3.1.6 A complete chain of custody record form is maintained.

5.8.3.2 Sample acceptance policy: the laboratory has a written sample acceptance policy that clearly outlines the circumstances under which samples shall be accepted or rejected. Data from any samples which do not meet the following criteria are flagged in an unambiguous manner clearly defining the nature and substance of the variation. This sample acceptance policy will be made available to sample collection personnel and shall include the following:

5.8.3.2.1 Proper, full, and complete documentation, which shall include sample identification, the location, date, and time of collection, collector's name, preservation type, sample type, and any special remarks concerning the sample.

5.8.3.2.2 Proper sample labeling to include unique identification and a labeling system for the samples with requirements concerning the durability of the labels (water resistant) and the use of indelible ink.

5.8.3.2.3 Use of appropriate sample containers.

5.8.3.2.4 Adherence to specified holding times.

5.8.3.2.5 Adequate sample volume. Sufficient sample volume must be available to perform the necessary tests.

5.8.3.2.6 Procedures to be used when samples show signs of damage, contamination, or inadequate preservation.

5.8.4 Source Molecular Corporation maintains procedures and facilities to avoid deterioration, contamination, loss or damage to sample during storage, handling, preparation, and testing. Handling instructions provided with the item are followed. Environmental conditions used for storage and conditioning of the items is maintained, monitored and recorded when necessary. Samples or portions thereof are secured and stored properly to protect the integrity of the sample or portions concerned when necessary.

5.8.4.1 Samples are stored according to the conditions specified by preservation protocols:

5.8.4.1.1 Samples that require thermal preservation are stored under refrigeration which is +/- 2 C of the specified preservation temperature unless method specific criteria exist. For samples with a specified storage temperature of 4 C, storage at a temperature form 1 C to 6 C shall be acceptable.

5.8.4.1.2 Samples shall be stored away from all standards, reagents, food, and other potentially contaminating sources. Samples shall be stored in such a manner to prevent cross contamination.

5.8.4.2 Sample fractions, extracts, leachates, and other sample preparation products shall be stored according to 5.8.4.1 or according to specifications in the test method.

5.8.4.2.1 The laboratory has SOP's for the disposal of samples, digestates, leachates, and extracts or other sample preparation products.

Responsibility

Laboratory Manager

References

Procedures

Receipt of Samples (SOP Q-4)
Sample Handling (SOP Q-15a)
PT Sample Handling (SOP Q-15b)

Quality Policy Manual

5.9 Assuring the Quality of Test Results

Policy

Source Molecular Corporation ensures the quality of test and calibration results is maintained.

Quality System Requirements

5.9.1 Quality control procedures exist for monitoring validity environmental tests undertaken. Resulting data is recorded to be able to detect trends using statistical techniques when practiceable. The monitoring will be planned and reviewed and may include:

5.9.1.1 Regular use of certified reference materials and/or internal quality control using secondary reference materials.

5.9.1.2 Participation in inter-laboratory comparison or proficiency testing programs.

5.9.1.3 Replicate tests using same or different methods.

5.9.1.4 Retesting of retained items.

5.9.1.5 Correlation of results for different characteristics of a sample.

5.9.2 Essential quality control procedures: the standards for any given test type shall assure that the applicable principles are addressed:

5.9.2.1 Detailed protocols are in place to monitor the following quality controls:

5.9.2.1.1 Positive and negative controls to monitor tests such as blanks, spikes, reference toxicants.

5.9.2.1.2 Tests to define the variability and/or repeatability of the laboratory results such as replicates.

5.9.2.1.3 Measures to assure the accuracy of the test method including calibration and/or continuing calibrations, use of certified reference materials, proficiency test samples, or other measures.

5.9.2.1.4 Measures to evaluate test method capability, such as limit of detection and limit of quantitation or range of applicability such as linearity.

5.9.2.1.5 Selection of appropriate formulae to reduce raw data to final results such as regression analysis, comparison to internal/external standard calculations, and statistical analyses.

5.9.2.1.6 Selection and use of reagents and standards of appropriate quality.

5.9.2.1.7 Measures to assure the selectivity of the test for its intended purpose.

5.9.2.1.8 Measures to assure constant and consistent test conditions, both instrumental and environmental, where required by the test method such as temperature, humidity, light, or specific instrument conditions.

5.9.2.2 All quality control measures are assessed and evaluated on an on-going basis, and quality control acceptance criteria are used to determine the usability of the data.

5.9.2.3 The laboratory has procedures for the development of acceptance/rejection criteria where no method or regulatory criteria exist. (See 5.8.3.2)

5.9.2.4 The quality control protocols specified by the laboratory's method manual (5.4.1.2) is followed. The laboratory ensures that the essential standards outlined in mandated methods or regulations are incorporated into method manuals.

5.9.3 Sterility checks and blanks: The laboratory demonstrates that the filtration equipment and filters, sample containers, media and reagents have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.

5.9.3.1 A sterility blank is analyzed for each lot of pre-prepared, ready-to-use medium and for each batch of medium prepared in the laboratory. This is done prior to first use of the medium.

5.9.3.2 For filtration technique, the laboratory conducts one beginning and one ending sterility check for each laboratory sterilization filtration unit used in a filtration series. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series. For pre-sterilized single use funnels a sterility check is performed on one funnel per lot. The filtration series is considered ended when more than 30 minutes elapses between successive filtrations. During a filtration series, filter funnels are rinsed with three 20-30 ml portions of sterile rinse water after each sample filtration. In addition, the laboratory inserts a sterility blank after every 10 samples or sanitize filtration units by UV light after each sample filtration.

5.9.3.3 For pour plate technique, sterility blanks of the medium are made by pouring, at a minimum, one uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared by the laboratory.

5.9.3.4 Sterility checks on sample containers are performed on at least one container for each lot of purchased, pre-sterilized containers. For containers prepared and sterilized in the laboratory, a sterility check is performed on one container per sterilized batch with non-selective growth media.

5.9.3.5 A sterility blank is performed on each batch of dilution water prepared in the laboratory and on each batch of pre-prepared, ready-to-use dilution water with non-selective growth media.

5.9.3.6 At least one filter from each new lot of membrane filters is checked for sterility with non-selective growth media.

5.9.4 Positive culture controls: each pre-prepared, ready-to-use lot of medium and each batch of medium prepared in the laboratory is tested with at least one pure culture of a known positive reaction. This is done prior to first use of the medium.

5.9.5 Negative Controls: each pre-prepared, ready-to-use lot of selective medium and each batch of selective medium prepared in the laboratory is analyzed with one or more known negative culture controls, i.e. non-target organisms, as appropriate to the method. This is done prior to first use of the medium.

5.9.6 Test Variability/Reproducibility: For test methods that specify colony counts such as membrane filter or plated media, duplicate counts are performed monthly on one positive sample, for each month that the test is performed. If the lab has two analysts, each analyst counts typical colonies on the same plate. Counts must be within 10% difference to be acceptable. If only one microbiology analyst is present, the same plate is counted twice by the analyst, with no more than 5% difference between the counts.

5.9.7 Test Performance:

5.9.7.1 All growth and recovery media are checked to assure that the target organism(s) respond in an acceptable and predictable manner.

5.9.7.2 To ensure that analysis results are accurate, target organism identity is verified as specified in the method, e.g. by use of the completed test, or by use of secondary verification tests such as a catalase test.

5.9.8 Quality of Standards, Reagents, and Media: the laboratory ensures that the quality of the reagents and media used is appropriate for the test concerned.

5.9.8.1 Culture media may be prepared from commercial dehydrated powders or may be purchased ready to use. Media may be prepared by the laboratory from basic ingredients when commercial media are not available or when it can be demonstrated that commercial media do not provide adequate results. Media prepared by the laboratory from basic ingredients is tested for performance prior to first use. Detailed testing criteria information is defined in either the laboratory's test methods, SOP's, Quality Manual, or similar documentation.

5.9.8.2 Reagents, commercial dehydrated powders and media are used within the shelf-life of the product and are documented according to 5.6.4.

5.9.8.3 Distilled water, deionized water or reverse-osmosis produced water from bactericidal and inhibitory substances are used in the preparation of media, solutions and buffers. The quality of the water is monitored for chlorine residual, specific conductance, and heterotrophic bacteria plate count monthly, when in use, when maintenance is performed on the water treatment system, or at startup after a period of disuse longer than one month. Analysis for metals and Bacterial Water Quality Test to determine presence of toxic agents or growth promoting substances is performed annually. Results of these analyses shall meet the specifications of the required method and records of analyses are maintained for five years. Exception: if documentation is supplied to show that the water source meets the criteria, as specified by the method, for Type 1 or type 2 reagent water.

5.9.8.4 Media, solutions, and reagents are prepared, used, and stored according to a documented procedure following manufacturer's instructions or the test method. Documentation for media prepared in the laboratory includes date of preparation, preparer's initials, type and amount of media prepared, manufacturer and lot number, final pH of the media, and expiration date. Documentation for media purchased pre-prepared, ready-to-use includes manufacturer, lot number, type and amount of media received, date of receipt, expiration date of the media, and pH of the media.

5.9.9 Selectivity: In order to ensure identity and traceability, reference cultures used for positive and negative controls are obtained from a recognized national collection, organization, or manufacturer recognized by the NELAP Accrediting Authority. Microorganisms may be single use preparations or cultures maintained by documented procedures that demonstrate the continued purity and viability of the organism.

5.9.9.1 Reference cultures may be revived if freeze dried or transferred from slants and subcultured once to provide reference stocks. The reference stocks are preserved by a technique which maintains the characteristics of the strains. Reference stocks are used to prepare working stocks for routine work. If reference stocks have been thawed, they must not be re-frozen and re-used.

5.9.9.2 Working stocks are not sequentially cultured more than five times and are not subcultured to replace reference stocks.

5.9.9.3 Annual or quarterly proficiency tests are performed to maintain quality control and to update Demonstration of Capability forms for all employees. Proficiency tests are either purchased from external PT programs or are spiked in-house using standards of known integrity.

Responsibility

Laboratory Manager

References

Personnel demonstration of capability notebook

Procedures

SOP Q-5

Quality Policy Manual 5.10 Reporting Results

Policy

Source Molecular Corporation ensures that the testing results are delivered properly. Source Molecular Corporation does not produce calibration reports.

Quality System Requirements

5.10.1 General: Results are reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test methods. Results reported in test report that includes all the information requested by the client and necessary for the interpretation of the results and methods used. For internal clients, or with written agreement with client, results may be reported in a simplified way. All information required by 5.10.2 to 5.10.4 shall be readily available in the lab that performed the testing.

5.10.1.1 The in-house laboratory is itself responsible for preparing the regulatory reports, or:

5.10.1.2 The laboratory provides information to another individual within the organization for preparation of regulatory reports. The facility management ensures that the appropriate report items are in the report to the regulatory authority if such information is required.

5.10.2 Test Reports: include the following information, unless the laboratory has a valid reason for not doing so, as indicated by 5.10.1.1 and 5.10.1.2.

5.10.2.1 Title.

5.10.2.2 Name and address of lab and location where testing was performed, if different from lab, and phone number with name of contact person for questions.

5.10.2.3 Unique identification of report, and on each page an identification to ensure that the page is recognized as a part of the report and a clear indication of the end of the report.

5.10.2.3.1 This requirement may be presented in several ways:

5.10.2.3.1.1 The total number of pages may be listed on the first page of the report as long as the subsequent pages are identified by the unique report identification and consecutive numbers.

5.10.2.3.1.2 Each page is identified with the unique report identification. The pages are identified as a number of total report pages.

5.10.2.3.2 Other methods of identifying the page in the report may be acceptable as long as it is clear to the reader that discrete pages are associated with a specific report, and that the report contains a specified number of pages.

5.10.2.4 Name and address of client and project name if applicable.

5.10.2.5 Identification of the method used.

5.10.2.6 Description, condition, and unambiguous identification of the sample(s), including client identification code.

5.10.2.7 Date of receipt of sample(s), where critical to validity and application of the results. Date and time of sample collection, Date(s) of performance of testing, and time of sample preparation and/or analysis if the required holding time for either activity is less than or equal to 72 hours.

5.10.2.8 Reference to the sampling plan and procedures.

5.10.2.9 Testing results with, where appropriate, the units of measurement, and any failures identified; identify whether data are calculated on a dry weight or wet weight basis; identify reporting units such as ug/L or mg/kg.

5.10.2.10 Name(s), functions, and signatures of personnel authorizing the test report, and date of issue.

5.10.2.11 A statement to the effect that the results relate only to the samples.

5.10.2.12 At the laboratory's discretion, a statement that the report shall not be reproduced except in full, without the written approval of the laboratory.

5.10.2.13 Certification that the test results meet all requirements of NELAC or provide reasons and/or/ justification if they do not.

5.10.3 Supplemental information for test reports:

5.10.3.1 Where necessary for the interpretation of results, the following shall be included in test reports:

5.10.3.1.1 Deviations from (e.g. failed quality control), additions to, or exclusions from the test method, and information on specific test conditions, such as environmental conditions and any non-standard conditions that may have affected the quality of results, including the use and definitions of data qualifiers.

5.10.3.1.2 Where quality system requirements are not met, a statement of compliance/ non-compliance with the requirements and/or specifications, including identification of test results derived from any sample that did not meet NELAC sample acceptance requirements such as improper container, holding time, or temperature.

5.10.3.1.3 Where applicable, a statement of the estimated uncertainty of measurement, particularly if the client's instruction so requires.

5.10.3.1.4 Where appropriate and needed, opinions and interpretations.

5.10.3.1.5 Additional information required by specific methods, clients or groups of clients.

5.10.3.1.6 Qualifications of numerical results with values outside the working range.

5.10.3.2 Test reports containing the results of sampling, when necessary, include for the interpretation of the test results:

5.10.3.2.1 Date of sampling

5.10.3.2.2 Unambiguous identification of the substance, material or product sampled, including name of manufacturer, model or type of designation and serial numbers as appropriate.

5.10.3.2.3 Location of sampling, including any diagrams, sketches and photographs.

5.10.3.2.4 Reference to sampling plan and procedure used.

5.10.3.2.5 Details of environmental conditions during sampling that may affect the interpretations of the test results.

5.10.3.2.6 Standard or specification for the sampling method or procedure, and deviations, additions to or exclusions from the specification concerned.

5.10.4 Opinions and Interpretations: When opinions and interpretations are included, the basis for such opinions and interpretations are documented and marked as such on the test reports.

5.10.5 Testing results obtained from subcontractors : Subcontracted test results are clearly identified on the test reports. The subcontractor shall report the results in writing or electronically. The laboratory shall make a copy of the subcontractor's report available to the client when requested by the client.

5.10.6 Electronic Transmission of Results: Results transmitted by telephone, telex, fax, or other electronic or electromagnetic means shall meet the requirements of this Standard and ensure that all reasonable steps are taken to preserve confidentiality.

5.10.7 Format of Reports and Certificates: Format is designed to accommodate each type of test carried out and to minimize the possibility of misunderstanding or misuse.

5.10.8 Amendments to Reports: Material amendments to the test reports after issue are made through further documents or data transfer which includes statement: "Supplement to Test Report, serial number…[or as otherwise identified]" or equivalent form of wording. Such amendments shall meet all the requirements of this Standard. When necessary to issue a complete new report, this will be uniquely identified and contain reference to the original that it replaces.

5.10.9 Data Reduction: The calculations, data reduction and statistical interpretations specified by each test method are followed.

Responsibility

Laboratory Directors

References

Procedures

Reporting (SOP Q-5)

Quality Policy Manual 5.11 Data Integrity

Policy

Source Molecular Corporation ensures data integrity is maintained.

Quality System Requirements

1.11.1 All laboratory staff involved in data storage and retrieval and report generation are trained in maintaining data integrity.
1.11.2 Signed data integrity documentation is kept on file for all laboratory employees undergoing said training.
1.11.3 Data integrity is monitored periodically and in depth.
1.11.4 Documentation of the data integrity procedure is kept on file.
1.11.5 Data integrity issues in the laboratory may be reported confidentially to management, who maintain confidentiality and a receptive environment.

Responsibility

Quality Manager

References

Procedures

Data Integrity SOP (Q-16)

Term	Definition
Accuracy	A measure of the difference (bias) between the Average of the readings from a measurement System and a corresponding benchmark or Master.
Benchmark Data	The results of an investigation to determine how Competitors and/or best-in-class Companies achieve their level of performance.
Capability	Capability is the total range of a stable process's inherent variation. It is determined using data from control charts. Reference "Fundamental Statistical Process Control".
Certified Registrars	Certified Registrars are qualified organizations certified by a national body (e.g., the Registrar Accreditation Board in the U.S.) to perform audits of the Quality System Requirements and to register the audited facility as meeting these requirements for a given commodity.
Conformity	The fulfillment of specified requirements.
Corrective Action Plan	A Corrective Action Plan is a plan for correcting a process or part quality issue.
Customer	The recipient of a product provided by the supplier.
Environment	Environment is all of the conditions surrounding and affecting manufacture and quality of a part or product.
Functional Verification	Functional Verification is testing to ensure the Part conforms to all customer and supplier engineering performance and material requirements.
Inspection	An activity such as measuring, examining, testing of gauging one or more characteristics of an entity and comparing the results with specified requirements in order to establish whether conformity is achieved for each characteristic.
Non-conformance	Non-conformance is product or material, which does not conform to the customer requirements or specifications.
Objective Evidence	Information, which can be proved true, based on facts obtained through observation, measurement, test or other means.
Ongoing Process	Ongoing process capability is a long term Capability measure of statistical process control, or process performance. Reference Fundamental SPC Reference Manual.
Organization	A company, corporation, firm, enterprise, or institution, or part thereof, whether incorporated or not, public or private, that has its own functions an administration.

Term	Definition
Parts Per Million (PPM)	PPM is a measure of process performance in terms of actual or projected non-conforming material. In general use, PPM defective is expressed as the proportion non-conforming (defective parts/total parts) times 1,000,000.
Preliminary Process Performance Studies	Preliminary Process Performance Studies are short-term studies conducted to obtain early information on the performance of new or revised processes relative to internal or customer requirements.
Procedure	A specified way to perform an activity. A documented procedure usually contains the purpose and scope of an activity; what shall be done and by whom; when, where and how it shall be done; what materials, equipment and documents shall be used; how it shall be controlled and recorded.
Process	A set of interrelated resources and activities, which transform input into, output.
Product	The result of activities or processes. Product includes service, hardware, processed materials, software, or a combination thereof. Product can be tangible or intangible or a combination of both Product can be intended or unintended.
Qualified	The status given to an entity when it has been demonstrated to be capable of fulfilling specified requirements.
Quality	The totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs.
Quality Manual	Quality Manual (also known as the Quality System Plan) is a document that describes the elements of the quality system used to assure customer requirements; needs and expectations are met. Quality manuals include responsibilities and authorities for each element of the quality system.
Quality Plan	A document identifying the specific quality practices, resources and sequence of activities relevant to a particular product, project, or contract.
Quality Planning	Quality Planning is a structured process for defining the methods (i.e., measurements, tests) that will be used in the production of a specific product or family of products (i.e., parts, materials.)
Quality Policy	The overall intentions and direction of an organization with regard to quality, as formally expressed by top management. The quality policy is one element of the corporate policy.

Quality Policy Manual Glossary

Term	Definition
Quality Record	A document which provides objective evidence of the extent of the fulfillment of the requirements for quality or the effectiveness of the operation of a quality system element.
Registered Suppliers/ Subcontractors	Registered Suppliers/Subcontractors are suppliers/subcontractors who have received third party ISO 9000 certification of the commodity supplied.
Registrar	A Registrar is a company that conducts quality system assessments to ISO 9000 Requirements.
Reliability	The probability that an item will continue to function at customer expectation levels at a measurement point, under specified environmental and duty cycle conditions.
Repair	The action taken on a nonconforming product so that is will fulfill the intended usage requirements, although it may not conform to originally specified requirements.
Rework	The action that will be taken on a nonconforming product so that is will fulfill the specified requirements.
Service	The results generated by activities at the interface between the supplier and the customer and by supplier internal activities, to meet customer needs.
Setup Verification	Formal review of process start-up (including equipment, tooling, material and conditions) to ensures that acceptable parts will be provided,
Special Characteristics	Product and process characteristics designated by the customer, including governmental regulatory and safety, and/or selected by the supplier through knowledge of the product and the process.
Special Processes	Production, installation and servicing processes requiring pre-qualification of process capability. This requirement is usually due to the inability to verify the process by subsequent inspection and testing of the product or where processing deficiencies may become apparent only after the product is in use.
Stability	The stability of a measurement system variation over time.
Statistical Process Control	The use of statistical techniques such as control charts to analyze a process or its output so as to take appropriate actions, which achieve and maintain a state of statistical control and improve the capability of the process.
Subcontractors	Subcontractors are defined as providers of materials, parts or services to a supplier.

Term	Definition
Supplier	The organization that provides a product to the customer.
Validation	Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled.
Verification	Confirmation by examination and provision of objective evidence that the specified requirements have been met.
Visual Controls	Visual controls are techniques for conveying information by visual means to observers so that everyone can understand whether current conditions are normal. Examples are floor markings, action boards, standardized work Charts and color-coding.

APPENDIX D – CHAIN-OF-CUSTODY FORM

COC# | **CHAIN OF CUSTODY** | **Page 1 of 1**

Project Name: Dix River MST

Project #: 5167C

Project Contact: Marcia L. Wooton

Collected By:

Third Rock Consultants, LLC

DRY WEATHER EVENT

- EXAMPLE -

* * Preservation Type									* * Preservation Code
ST									ST = Na2S203 & Ice

Requested Analysis	
Total Coliform* E.coli	*Report Atypical & Typical Colonies

Sample I.D.		Location	Sample Collection Date	Sample Collection Time	Grab / Comp	Filt'd Y/N	4oz Sterile Plastic									Lab Sample #	Comments
TRC_MC1LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC2LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC3LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC4LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC5LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC6LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC7LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
TRC_MC8LP01-YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										
MCDUPLICATE #YYYYMM	DD	Hanging Fork/McKinney Branch			G	N	1										

Relinquished By:	Date / Time:	Received By:	Date / Time:

Laboratory: ADD hand-written "date", highlighted in yellow, to sample id (without any spaces).

Properly Preserved: (Yes / No) Headspace: (Yes / No)

COC Seals Intact: (Yes / No / NA) Bottles Intact: (Yes / No)

Temp. Upon Receipt (C) _____ By:_____

Field Document Attached: (Yes / No)

mlw 07/19/07 Update

APPENDIX E – ANALYTICAL LABORATORY REPORT EXAMPLES

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

Cow Bacteroidetes ID™

Detection of the fecal *Bacteroidetes* Cattle Gene Biomarker for Cattle Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: XYZ Municipal Beach
Submitter #'s: 675, 676, 677 and 678
Source Molecular #'s: SM 0625, SM 0626, SM 0627 and SM 0628
Samples Received: May 25, 2004
Date Reported: June 02, 2004

SM #	Client #	DNA Analytical Results
SM 0625	675	Cattle Gene Biomarker Detected
SM 0626	676	Negative
SM 0627	677	Cattle Gene Biomarker Detected
SM 0628	678	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 676 (Our Ref: SM 0626) and 678 (Our Ref: SM 0628) tested negative for the fecal *Bacteroidetes* cattle gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have cattle contamination. In order to strengthen the result, a negative sample should be analyzed further for cattle fecal contamination with other DNA analytical tests such as the Cow Enterococcus ID™ and Cow Fecal Virus ID™ services.

Samples 675 (Our Ref: SM 0625) and 677 (Our Ref: SM 0627) tested positive for the fecal *Bacteroidetes* cattle gene biomarker suggesting that cattle 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 Cow 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 detected in cattle. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in cattle.[2,3,5] As such, these bacterial strains can be used as indicators of cattle fecal contamination.

One of the advantages of the Cow 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 cattle gene biomarker from the *Bacteroides* and *Prevotella* genus.

These banding patterns confirm or negate the presence of the fecal *Bacteroidetes* cattle gene biomarker. As such, the banding patterns can be a good indicator of cattle fecal contamination. Nonetheless, in order to strengthen the validity of the results, the Cow Bacteroidetes ID™ service should be combined with other DNA analytical services such as the Cow Enterococcus ID™ and Cow Fecal Virus ID™ services since the fecal *Bacteroidetes* cattle gene biomarker has been detected occasionally in other ruminants.[3]

[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.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[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] Fogarty, Lisa R., Voytek, Mary **A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

[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

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

Cow Bacteroidetes "Quantification" ID™

Detection and Quantification of the Fecal *Bacteroidetes* Cattle Gene Biomarker for Cattle Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: XYZ Municipal Beach
Submitter #'s: 675, 676, 677 and 678
Source Molecular #'s: SM 0625, SM 0626, SM 0627 and SM 0628
Samples Received: July 17, 2006
Date Reported: July 23, 2006

SM #	Client #	Total *Bacteroidetes* Quantified*,7,8	Cattle Fecal *Bacteroidetes* Quantified*,7,8	DNA Analytical Results
SM 0625	675	5.85×10^{10}	BDL ‡	Negative ‡
SM 0626	676	2.50×10^{10}	8.95×10^{5}	Cattle Gene Biomarker Detected
SM 0627	677	4.66×10^{9}	BDL ‡	Negative ‡
SM 0628	678	2.54×10^{10}	BDL ‡	Negative ‡

*** Number given is the copy number of the cattle *Bacteroides* 16S RNA marker per copy no./ml of DNA extract - see DNA Analytical Method Explanation.**
‡ Below Detection Limit. Detection limit is < 2,000 copy no./ml of DNA extract.

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 Comments
Submitter: XYZ Municipal Beach
Report Date: July 23, 2006

The submitted water samples were filtered for fecal *Bacteroidetes*. Afterwards, the filters were eluted in a buffer. The buffer was centrifuged and DNA was extracted from the resultant pellet. qPCR (i.e.: real-time quantitative PCR) targeting total fecal *Bacteroidetes* and the fecal *Bacteroidetes* cattle gene biomaker was performed on the DNA extract. 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.

The results for samples 675 (Our Ref: SM 0625), 677 (Our Ref: SM 0627) and 678 (Our Ref: SM 0628) were below the detection limits of the real-time qPCR assay. They were therefore classified as negative for the fecal *Bacteroidetes* cattle gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have cattle contamination. In order to strengthen the result, a negative sample should be analyzed further for cattle fecal contamination with other DNA analytical tests such as the Cow Enterococcus ID™ and Cow Fecal Virus ID™ services. On the other hand, one can infer the presence of animal sources of fecal pollution since generic forms of fecal *Bacteroidetes* were found present in the negative sample.

Preliminary Interpretation of Positive Result

Sample 676 (Our Ref: SM 0626) tested positive for the fecal *Bacteroidetes* cattle gene biomarker suggesting that cattle fecal contamination is present in this water sample. Using real-time quantitative PCR DNA analytical technology (qPCR), the fecal *Bacteroidetes* with the cattle gene marker was quantified and compared to the total fecal *Bacteroidetes* population. The fecal *Bacteroidetes* with the cattle gene marker gave a ratio of 0.0036% of the total fecal *Bacteroidetes* population. It is important to take into account the context of the sample when interpreting the percentage provided.

Our preliminary interpretation suggests that cattle fecal sources of contamination are a minor component of the positive sample. Using our internal ratios, the cattle fecal pollution would seem to be less than 1% of the overall fecal pollution of the sample. The client is encouraged nonetheless to submit additional samples from this site both during wet and dry events to get a better understanding of the cattle fecal pollution contribution. Furthermore, a baseline of cow dung samples from the surrounding area of study would help gain a better understanding of the percentage of the cattle marker present within the geographic region. A more precise interpretation would be available to the client with the submittal of such baseline samples. The client is also encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the positive result.

DNA Analytical Method Explanation

The 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.

The copy number of the *Bacteroides* cattle marker was determined using in-house modifications of the primer and probe sequences published in peer-reviewed literature.[2,3,6,7,8] Amplifications were run on an ABI Prism 7300. The final reaction volume (25ul) contained 2.5ul of sample extract, 900nm of forward and reverse primers, 200nM Taqman minor groove binder probe and 1X Applied Biosystems TaqMan PCR Master Mix. Thermal cycling parameters were 2 min at 50 deg.C, 10 min at 95 deg.C followed by 40 cycles of 30 s at 95 deg.C and 60 deg. C for 1 min. All assays were run in triplicate. Absolute quantification was achieved by generating standard curves from serial dilutions of synthesized final amplicon target sequence.

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 Cow Bacteroidetes "Quantification" ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[4,5] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately found in cattle.[2,3,5,6,7,8] Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found to be specific to cattle.[2,3,5,6,7,8] As such, these bacterial strains can be used as indicators of cattle fecal contamination.

One of the advantages of the Cow Bacteroidetes "Quantification" 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 analysis.

Real-time quantitative PCR (qPCR) adds a variant to the PCR step by inserting of a fluorescent probe within the primer set. This fluorescent probe serves as a molecular beacon for the quantification step. During each PCR cycle, real-time quantification PCR monitors the fluorescence emitted during the reaction. This is done in "real-time" during the first PCR cycles as a way to quantify the targeted gene.

The Cow Bacteroidetes "Quantification" ID™ service uses real-time quantification PCR to simultaneously confirm and quantify total fecal *Bacteroidetes* and the cow specific *Bacteroides* 16S rRNA genetic marker.[2,3,6,7,8] This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. The results are presented on a computer screen and printout thus avoiding ambiguities in interpretation.

This data should serve only as a preliminary indicator of the relative cattle pollution in the water sample. The context of the sample should be taken into account when interpreting the amount of the fecal *Bacteroidetes* cattle gene biomaker. Submitting one or more cattle reference samples from the geographic area of interest helps establish a baseline biomarker level. With these baseline numbers, the client can make a more meaningful interpretation of the data. To strengthen the validity of the results, the Cow Bacteroidetes "Quantification" ID™ service should also be combined with other DNA analytical services such as the Cow Enterococcus ID™ and Cow 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.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[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] Fogarty, Lisa R., Voytek, Mary **A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

[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.

[7] Reischer, Georg H., Kasper, David C., Steinborn, Ralf, Mach, Robert L., Farnleitner, Andreas H. **Quantitative PCR Method for Sensitive Detection of Ruminant Fecal Pollution in Freshwater and Evaluation of This Method in Alpine Karstic Regions** Appl. Environ. Microbiol. 2006 72: 5610-5614.

[8] Layton, Alice, McKay, Larry, Williams, Dan, Garrett, Victoria, Gentry, Randall, Sayler, Gary **Development of Bacteroides 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water** Appl. Environ. Microbiol. 2006 72: 4214-4224

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

Cow Enterococcus ID™

Detection of the *Enterococcus hirae* Cattle Gene Biomarker for Cattle 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, 2006
Date Reported: May 23, 2006

SM #	Client #	Enterococci (CFU/100mL)^7	DNA Analytical Results
SM 0125	775	45	Cattle Gene Biomarker Detected
SM 0126	776	150	Negative
SM 0127	777	255	Cattle Gene Biomarker Detected
SM 0128	778	15	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 hirae* cattle gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have cattle contamination. In order to strengthen the result, a negative sample should be analyzed further for cattle fecal contamination with other DNA analytical tests such as the Cow Bacteroidetes ID™ and Cow Fecal Virus ID™ services.

Samples 775 (Our Ref: SM 0125) and 777 (Our Ref: SM 0127) tested positive for the *Enterococcus hirae* cattle gene biomarker suggesting that cattle 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 0.45 micron membrane filters. The filters were placed on mEnterococcus media supplemented with indoxyl substrate and the plates were incubated for 24 hours similar 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^{o}C for 15 minutes (to lyse cells and activate polymerase), followed by 35 cycles of 94^{o}C for 1 minute, 55^{o}C for 1 minute, and 72^{o}C for 1 minute and a final extension at 72^{o}C for 5 minutes. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Cambrex, 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^{o}C and 45^{o}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.[1,2,3]

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.[4,5] 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 Cow Enterococcus ID^{TM} service is designed around the principle that certain DNA sequences contained within strains of the *Enterococcus* genus are specific to cattle. These *Enterococci* sequences can be used as indicators of cattle fecal contamination.[6] Strains of *Enterococcus hirae* and *Enterococcus mundtii* have been shown to be from cattle and other ruminant sources.[6] The Cow Enterococcus ID^{TM} service targets the cattle gene biomarker in *Enterococcus hirae*.

One of the advantages of the Cow Enterococcus ID^{TM} 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.

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 cattle gene biomarker from *Enterococcus hirae.*

These banding patterns confirm or negate the presence of the *Enterococci* cattle gene biomarker. As such, the banding patterns provide a reliable indicator of cattle fecal contamination. To strengthen the validity of the results, the Cow Enterococcus ID™ service should be combined with other DNA analytical services such as the Cow Bacteroidetes ID™ and Cow 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] 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.

[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] Soule, Marilyn, Kuhn, Edward, Loge, Frank, Gay, John, Call, Douglas R. **Using DNA Microarrays To Identify Library-Independent Markers for Bacterial Source Tracking** Appl. Environ. Microbiol. 2006 72: 1843-1851.

[7] **EPA Method 1600: Membrane Filter Test Method for Enterococci In Water (1997).**

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

Cow Enterococcus "Quantification" ID™

Detection and Quantification of the *Enterococcus hirae* Cattle Gene Biomarker for Cattle Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: ABC Beach Park
Submitter #'s: 875, 876, 877 and 878
Source Molecular #'s: SM 0825, SM 0826, SM 0827 and SM 0828
Samples Received: August 14, 2006
Date Reported: August 17, 2006

SM #	Client #	Entero-cocci (CFU/100 mL)***	Total *E. faecium* Quantified*	Total *E. hirae* Cattle Biomarker Quantified*	DNA Analytical Results
SM 0825	875	400	2.95×10^7	BDL**	Negative **
SM 0826	876	1,500	1.15×10^8	3.55×10^4	Cattle Gene Biomarker Detected
SM 0827	877	1,400	2.25×10^8	BDL**	Negative **
SM 0828	878	5,400	4.65×10^8	BDL**	Negative **

* After 24 hours of incubation. Total is copy no./ml of extract. See laboratory comments.
** Detection limit is <1,500 copy no./ml of DNA extract.
*** EPA Method 1600: Membrane Filter Test Method for Enterococci In Water (1997).

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 Comments
Submitter: ABC Beach Park
Report Date: August 17, 2006

The submitted water samples were filtered and incubated for 24 hours. **Please note that the *Enterococci* numbers given in the table on the next page are after cultivation.** Afterwards, the filters were eluted in a buffer. The buffer was centrifuged and DNA was extracted from the resultant pellet. qPCR (i.e.: real-time quantitative PCR) targeting total *E. faecium* and the *E. hirae* cattle gene biomaker was performed on the DNA extract.

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.

All the samples in this report except sample 876 (SM 0826) tested negative (i.e. below the detection limit) for the *Enterococcus hirae* cattle gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have cattle contamination. In order to strengthen the result, a negative sample should be analyzed further for cattle fecal contamination with other DNA analytical tests such as the Cow Bacteroidetes ID™ and Cow Fecal Virus ID™ services. On the other hand, one can infer the presence of animal sources of fecal pollution since generic forms of *Enterococcus faecium* were found present in the negative samples.

Preliminary Interpretation of Positive Result

Sample 876 (SM 0826) tested positive for the *Enterococcus hirae* cattle gene biomarker suggesting that cattle fecal contamination is present in this water sample. Using real-time quantitative PCR DNA analytical technology (qPCR), the *E. hirae* with the cattle gene marker was quantified and compared to the total *E. faecium* population. The *E. hirae* with the cattle gene marker gave ratio of 0.031% of the total *E. faecium* population. It is important to take into account the context of the sample when interpreting the percentage provided.

Our preliminary interpretation suggests that cattle fecal sources of contamination are a minor component of the positive sample. Using our internal ratios, the cattle fecal pollution would seem to be less than 1% of the overall fecal pollution of the sample. The client is encouraged nonetheless to submit additional samples from this site both during wet and dry events to get a better understanding of the cattle fecal pollution contribution. Furthermore, a baseline of cow dung samples from the surrounding area of study would help gain a better understanding of the percentage of the cattle marker present within the geographic region. A more precise interpretation would be available to the client with the submittal of such baseline samples. The client is also encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the positive result.

DNA Analytical Method Explanation

100 ml of water was filtered through 0.45 micron membrane filters and placed on mEI agar. The samples were incubated for 24 hours. Each filter was removed, placed in buffer and vortexed vigorously. Once the buffer was spun to pellet the bacteria, the supernatant was removed and the pellet was resuspended in a small volume of water. DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturer's instructions.

2.5 micro-liter aliquots of purified DNA extraction were used directly as template for subsequent qPCR reactions. All assays were run on an ABI 7300 under the following thermal cycling conditions: 50°C for 2 minutes and 95°C for 10 minutes followed by 40 cycles of 95°C for 10 seconds and 57°C for 1 minute. Default data collection parameters were employed. The Taqman master mix supplied by Applied Biosystems was used with the forward and reverse primers added to a final concentration of 900nM and the probe added to a final concentration of 0.125uM with a 25ul final total reaction volume.

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.[1,2,3]

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.[4,5] 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 Cow Enterococcus "Quantification" ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Enterococcus* genus are specific to cattle. These *Enterococci* sequences can be used as indicators of cattle fecal contamination.[6] Strains of *Enterococcus hirae* and *Enterococcus mundtii* have been shown to be from cattle and other ruminant sources.[6] The Cow Enterococcus "Quantification" ID™ service targets the cattle gene biomarker in *Enterococcus hirae*.

One of the advantages of the Cow Enterococcus "Quantification" 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.

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 analysis.

Real-time quantitative PCR (qPCR) adds a variant to the PCR step by inserting of a fluorescent probe within the primer set. This fluorescent probe serves as a molecular beacon for the quantification step. During each PCR cycle, real-time quantification PCR monitors the fluorescence emitted during the reaction. This is done in "real-time" during the first PCR cycles as a way to quantify the targeted gene.

The Cow Enterococcus "Quantification" ID™ service uses real-time quantification PCR to simultaneously confirm and quantify total *Enterococcus faecium*, which is used as an indicator of total *Enterococcus* loading, and the cattle gene biomaker in *E. hirae*. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. The results are presented on a computer screen and printout thus avoiding ambiguities in interpretation.

This data should serve only as a preliminary indicator of the relative cattle pollution in the water sample. The context of the sample should be taken into account when interpreting the amount of the *Enterococcus hirae* cattle gene biomaker. Submitting one or more cattle reference samples from the geographic area of interest helps establish a baseline biomarker level. With these baseline numbers, the client can make a more meaningful interpretation of the data. To strengthen the validity of the results, the Cow Enterococcus "Quantification" ID™ service should also be combined with other DNA analytical services such as the Cow Bacteroidetes ID™ and Cow 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] 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.

[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] Soule, Marilyn, Kuhn, Edward, Loge, Frank, Gay, John, Call, Douglas R. **Using DNA Microarrays To Identify Library-Independent Markers for Bacterial Source Tracking** Appl. Environ. Microbiol. 2006 72: 1843-1851.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

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

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.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[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

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

Human Bacteroidetes "Quantification" ID™

Detection and Quantification of the Fecal *Bacteroidetes* Human Gene Biomarker for Human Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: XYZ Municipal Beach
Submitter #'s: 675, 676, 677 and 678
Source Molecular #'s: SM 0625, SM 0626, SM 0627 and SM 0628
Samples Received: February 17, 2005
Date Reported: February 23, 2005

SM #	Client #	Total Fecal *Bacteroidetes* Quantified*,6	Human Fecal *Bacteroidetes* Quantified*,7	DNA Analytical Results
SM 0625	675	5.85×10^{10}	3.45×10^{4}	Human Gene Biomarker Detected
SM 0626	676	7.50×10^{10}	6.55×10^{4}	Human Gene Biomarker Detected
SM 0627	677	4.66×10^{9}	BDL ‡	Negative ‡
SM 0628	678	2.54×10^{10}	BDL ‡	Negative ‡

*** Number given is the copy number of the human *Bacteroides* 16S RNA marker per liter of water - see DNA Analytical Method Explanation, Reference 7.**

‡ Below Detection Limit. Detection limit is < 500 copy number per liter of water.

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 Comments
Submitter: XYZ Municipal Beach
Report Date: February 23, 2005

The submitted water samples were filtered for fecal *Bacteroidetes*. Afterwards, the filters were eluted in a buffer. The buffer was centrifuged and DNA was extracted from the resultant pellet. qPCR (i.e.: real-time quantitative PCR) targeting the fecal *Bacteroidetes* human gene biomaker was performed on the DNA extract. 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.

The results for samples 677 (Our Ref: SM 0627) and 678 (Our Ref: SM 0628) were below the detection limits of the real-time qPCR assay. They were therefore classified as 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. On the other hand, one can infer the presence of animal sources of fecal pollution since generic forms of fecal *Bacteroidetes* were found present in the negative samples

Preliminary Interpretation of Positive Results

Samples 675 (Our Ref: SM 0625) and 676 (Our Ref: SM 0626) tested positive for the fecal *Bacteroidetes* human gene biomarker suggesting that human fecal contamination is present in these water samples. Using real-time quantitative PCR DNA analytical technology (qPCR), the amount of the fecal *Bacteroidetes* with the human gene marker (shown in the table on the previous page) was calculated.

It is important to take into account the context of the sample when interpreting the amount provided. It is also recommended to conduct other DNA analytical tests such as the services mentioned above to further confirm the results.

Our preliminary interpretation suggests that human fecal sources of contamination are a minor component of the positive samples. Using our internal ratios (i.e. human fecal *Bacteroidetes* / total *Bacteroidetes*), the human fecal pollution would seem to be less than 1% of the overall fecal pollution of the samples. The client is encouraged nonetheless to submit additional samples from these sites both during wet and dry events to get a better understanding of the human fecal pollution contribution. Furthermore, a baseline of raw sewage samples from the surrounding wastewater facilities and/or septic systems would help gain a better understanding the fecal *Bacteroidetes* human gene biomaker present within the local population. A more precise interpretation would be available to the client with the submittal of such baseline samples. The client is also encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the positive 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.

The copy number of the *Bacteroides* human marker was determined using the primer sequences described by Seurinck et al.[7] Amplifications were run on an ABI Prism 7300. The final reaction volume (25ul) contained 2.5ul of sample extract, 250nM of forward and reverse primers and 1X Applied Biosystems SyBr Green PCR Master Mix. Thermal cycling parameters were 2 min at 50 deg.C, 10 min at 95 deg.C followed by 40 cycles of 30 s at 95 deg.C, 53 deg. C for 1 min. and 60 deg. C for 1 min. All assays were run in triplicate. Absolute quantification was achieved by generating standard curves from serial dilutions of synthesized final amplicon target sequence.

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 "Quantification" 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 "Quantification" 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 analysis.

Real-time quantitative PCR (qPCR) adds a variant to the PCR step by inserting of a fluorescent probe within the primer set. This fluorescent probe serves as a molecular beacon for the quantification step. During each PCR cycle, real-time quantification PCR monitors the fluorescence emitted during the reaction. This is done in "real-time" during the first PCR cycles as a way to quantify the targeted gene.

The Human Bacteroidetes "Quantification" ID™ service uses real-time quantification PCR to simultaneously confirm and quantify total fecal *Bacteroidetes* and the human-specific HF183 *Bacteroides* 16S rRNA genetic marker.[6,7] This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. The results are presented on a computer screen and printout thus avoiding ambiguities in interpretation.

Once each targeted gene is quantified, a relative percentage can be calculated. As such, it has been hypothesized that relative levels of human pollution can be interpreted by the proportion of the human gene biomaker found in fecal *Bacteroidetes* relative to the total population of fecal *Bacteroidetes* in the water sample.[6,7] Nonetheless this data should serve only as a preliminary indicator of relative human pollution in the water sample. Furthermore, the context of the sample should be taken into account when interpreting the relative percentage provided. To strengthen the validity of the results, the Human Bacteroidetes "Quantification" ID™ service should also 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.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[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.

[7] Seurinck, S., T. Defoirdt, W. Verstraete, and S. D. Siciliano. **Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human fecal pollution in freshwater.** Environmental Microbiology 2005 7:2 p. 249.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

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

SM #	Client #	Enterococci (CFU/100mL)^{7}	DNA Analytical Results
SM 0125	775	45	Human Gene Biomarker Detected
SM 0126	776	150	Negative
SM 0127	777	255	Human Gene Biomarker Detected
SM 0128	778	15	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. 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

For each sample, 100 ml of water was filtered through a 0.45 micron membrane filter. The filter was placed on mEnterococcus media supplemented with indoxyl substrate and the plate was incubated for 24 hours similar 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^{o}C for 15 minutes (to lyse cells and activate polymerase), followed by 35 cycles of 94^{o}C for 1 minute, 55^{o}C for 1 minute, and 72^{o}C for 1 minute and a final extension at 72^{o}C for 5 minutes. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Cambrex, 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^{o}C and 45^{o}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^{TM} 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^{TM} service targets the esp human gene biomarker in *Enterococcus faecium*.[6]

One of the advantages of the Human Enterococcus ID^{TM} 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. 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 *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).**

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

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

Human Enterococcus "Quantification" ID™

Detection and Quantification of the *Enterococcus faecium* esp Human Gene Biomarker for Human Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: ABC Beach Park
Submitter #'s: 875, 876, 877 and 878
Source Molecular #'s: SM 0825, SM 0826, SM 0827 and SM 0828
Samples Received: September 14, 2004
Date Reported: September 17, 2004

SM #	Client #	Entero-cocci (CFU/100 mL)***	Total *E. faecium* Quantified*	Total *E. faecium* esp Human Biomarker Quantified*	DNA Analytical Results
SM 0825	875	400	2.95×10^7	BDL**	Negative **
SM 0826	876	1,500	1.15×10^8	3.55×10^4	Human Gene Biomarker Detected
SM 0827	877	1,400	2.25×10^8	BDL**	Negative **
SM 0828	878	5,400	4.65×10^8	BDL**	Negative **

*** After 24 hours of incubation. Total is copy no./ml of extract. See laboratory comments.**
**** Below Detection Limit. Detection limit is < 2,000 copy no./ml of DNA extract.**
***** EPA Method 1600 (modified): Membrane Filter Test Method for Enterococci In Water (1997).**

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 Comments
Submitter: ABC Beach Park
Report Date: September 17, 2004

The submitted water samples were filtered and incubated 24 hours. **Please note that the *E. faecium* numbers given in the table on the next page are after cultivation.** Afterwards, the filters were eluted in a buffer. The buffer was centrifuged and DNA was extracted from the resultant pellet. qPCR (i.e.: real-time quantitative PCR) targeting total *E. faecium* and the *E. faecium* esp human gene biomaker was performed on the DNA extract.

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.

All the samples in this report except sample 876 (SM 0826) tested negative (i.e. below the detection limit) 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. 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. On the other hand, one can infer the presence of animal sources of fecal pollution since generic forms of *Enterococcus faecium* were found present in the negative samples.

Preliminary Interpretation of Positive Result

Sample 876 (SM 0826) tested positive for the *Enterococcus faceium* esp human gene biomarker suggesting that human fecal contamination is present in this water sample. Using real-time quantitative PCR DNA analytical technology (qPCR), the *E. faecium* with the esp human gene marker was quantified and compared to the total *E. faecium* population. The *E. faecium* with the esp human gene marker was found in 0.02% of the total *E. faecium* population.

Internal tests in our laboratory have shown that the human esp marker can be present in 1% to 3.5% in the *E. faecium* population of a raw sewage sample in North America. Diluted samples, such as stormwater runoff have also shown to have similar ratios (i.e. internal tests and client's reference sample) if raw sewage is an important source of the contamination. For combined sewer overflows (CSO), the ratios from internal laboratory tests indicate a 10-fold dilution; therefore if one is monitoring CSO's, one should take into account this dilution factor. Consequently, it is important to take into account the context of the sample when interpreting the percentage provided.

Our preliminary interpretation suggests that human fecal sources of contamination are a minor component of the positive sample. Using our internal ratios, the human fecal pollution would seem to be less than 1% to 5% of the overall fecal pollution of the sample. The client is encouraged nonetheless to submit additional samples from this site both during wet and dry events to get a better understanding of the human fecal pollution contribution. Furthermore, a baseline of raw sewage samples from the surrounding wastewater facilities and/or septic systems would help gain a better understanding of the percentage of the esp human marker present within the local population. A more precise interpretation would be available to the client with the submittal of such baseline samples. The client is also encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the positive result.

DNA Analytical Method Explanation

100 ml of water was filtered through 0.45 micron membrane filters and placed on mEI agar. The samples were incubated for 24 hours. Each filter was removed, placed in buffer and vortexed vigorously. Once the buffer was spun to pellet the bacteria, the supernatant was removed and the pellet was resuspended in a small volume of water. DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturer's instructions.

2.5 micro-liter aliquots of purified DNA extraction were used directly as template for subsequent qPCR reactions. All assays were run on an ABI 7300 under the following thermal cycling conditions: 50°C for 2 minutes and 95°C for 10 minutes followed by 40 cycles of 95°C for 10 seconds and 57°C for 1 minute. Default data collection parameters were employed. The Taqman master mix supplied by Applied Biosystems was used with the forward and reverse primers added to a final concentration of 900nM and the probe added to a final concentration of 0.125uM with a 25ul final total reaction volume.

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 "Quantification" 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 "Quantification" ID™ service targets the esp human gene biomarker in *Enterococcus faecium*.[6]

One of the advantages of the Human Enterococcus "Quantification" 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.

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 analysis.

Real-time quantitative PCR (qPCR) adds a variant to the PCR step by inserting of a fluorescent probe within the primer set. This fluorescent probe serves as a molecular beacon for the quantification step. During each PCR cycle, real-time quantification PCR monitors the fluorescence emitted during the reaction. This is done in "real-time" during the first PCR cycles as a way to quantify the targeted gene.

The Human Enterococcus "Quantification" ID™ service uses real-time quantification PCR to simultaneously confirm and quantify total *Enterococcus faecium* and the esp human gene biomaker in *E. faecium*. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. The results are presented on a computer screen and printout thus avoiding ambiguities in interpretation.

Once each targeted gene is quantified, a relative percentage can be calculated. As such, it has been hypothesized that relative levels of human pollution can be interpreted by the proportion of the esp human gene biomaker found in *E. faecium* relative to the total population of *E. faecium* in the water sample.[6] Nonetheless this data should serve only as a preliminary indicator of relative human pollution in the water sample. Furthermore, the context of the sample should be taken into account when interpreting the relative percentage provided. To strengthen the validity of the results, the Human Enterococcus "Quantification" ID™ service should also 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.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.

APPENDIX F – PREPRINTED LABEL EXAMPLE

Third Rock Consultants, LLC
Project: Dix River MST
Site: Hanging Fork/McKinney Branch
Sample ID: TRC_MC1LPO1-_ _ _ _ _ _ _ _ _
Date/Time Collected: _ _-_ _-_ _ / _ _ _ _
Parameter(s): Total Coliform and E. coli

APPENDIX G – MICROBIOLOGY GUIDE DOCUMENT AND QUALITY CONTROL PROCEDURES

Version: 2
Revision: 2
Date accepted: 07/11/07 by Dr. T.M. Scott

Microbiology Guide Document and Quality Control Procedures

(MGD-1)

Source Molecular Corporation

4842 SW 74th Court
Miami, FL 33155

Policy Statement

The management and personnel of Source Molecular Corporation are committed to good professional practice and to provide analytical services in compliance with stringent standards of quality. All analyses performed by said Laboratory shall be in accordance with established assurance practices and specific, written testing procedures. All employees shall be familiar with their responsibilities under the program and implement the

policies and procedures in their work. The quality manual shall be readily available to all employees and maintained up-to-date along with quality documentation.

Objectives

1. Test results shall be of known quality
2. The precision and accuracy of all test data shall be determined
3. Data acquisition, transfer and report preparation steps shall be documented
4. All reports shall be reviewed for completeness and conformance to the quality system program by the appropriate department head, the lab director or the lab manager.
5. Raw data, quality control data and reports shall be stored and retrievable
6. Sample receiving shall ensure that the Laboratory sample acceptance policy is met.
7. Samples shall be retrievable until disposal is called for
8. All operations shall be performed in accordance with and in conformance to detailed, documented standard operating procedures.

Methods and Procedures

1.0 Reference Cultures. All reference cultures of Microorganisms for positive and negative controls and routine laboratory analyses are purchased from the American Type Culture Collection (ATCC). ATCC information is logged into the reference culture notebook and cultures are stored according to supplier's specifications.

2.0 Revival of Reference Cultures. Reference cultures are stored according to supplier's specifications. Frozen cultures are revived and subcultured only once to provide reference stocks. Reference stocks are only revived once to maintain working stocks. Reference cultures are only replaced directly from supplier (e.g. ATCC).

3.0 Working Cultures. Working stocks are preserved by a method deemed appropriate that maintains desired characteristics of the reference organism. Working stocks are not thawed and reused. Working Cultures do not replace reference cultures.

3.1 Bacterial cultures. Maintained on solid media plates (non-selective). Prior to analyses, cultures are streaked onto selective and differential medium to assess desired characteristics. Bacterial cultures are only subcultured 5 times before obtaining another Reference Culture.

3.2 Mammalian Tissue Culture. Stocks are maintained in culture flasks and passed no more than 300 times. After 300 passages, stocks are destroyed and reordered from ATCC.

3.3 Viral Cultures. Viral cultures are kept frozen at –20 oC in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS).

4.0 Cleaning and disinfection of work areas. All floors and work surfaces are cleaned and disinfected weekly with a commercially available cleaning/disinfection agent. Routine surface area disinfection is accomplished by spraying work areas with an aqueous solution containing 70% ethanol and wiping with paper towels. Flooring in the laboratory is tile and all work surfaces are sealed formica. Dusting is performed weekly.

5.0 Food and Drink in the Laboratory. All food and drink is prohibited in the laboratory. Food and drink is only allowed in offices and reception areas.

6.0 Temperature monitoring and recording. All incubators, refrigerators, freezers, and water baths are monitored by NIST traceable thermometers. Temperature logs are kept at the equipment and when log is full it is filed in equipment notebook under the appropriate item. Data on traceability of thermometers is available in the equipment notebook. Temperatures are read and recorded to most precise scale measured by the thermometer.

7.0 Equipment calibration and performance.

7.1 Thermometers. All thermometers are replaced annually by NIST-traceable products.

7.2 Autoclave. Autoclaves are monitored for maximum temperature on each run and all data is recorded on autoclave log sheet with each run. Temperature measurements are made from different quadrants in succession and the thermometer location is reference on the log sheet. All items to be autoclaved are appropriately labeled and marked with temperature tape. Spore tests are performed at least monthly and results are logged on autoclave performance sheet. Autoclave thermometers are replaced annually. Autoclave timing device is checked quarterly by comparing to atomic clock at www.nist.gov. All completed log sheets and performance sheets are filed in the equipment notebook.

7.3 Pipets. All pipets and volumetric equipment is calibrated every six months by an outside vendor. Results from calibrations are recorded in the equipment notebook. Each lot number of disposable pipets and pipet tips is checked for accuracy by weighing amount of discharged purified water on an analytical balance (eg. 10 ml = 10 g). All pipets are calibrated monthly in-

house by this method and results are recorded on pipet calibration log sheets. Completed log sheets are filed in the equipment notebook.

7.4 UV disinfection instruments. UV bulbs are not used for routine surface disinfection. UV bulb in the Biosafety cabinet is checked quarterly for biocidal activity by Plate Count Agar spread plates.

7.5 pH Meters. All pH meters are calibrated using two separate lots of NIST traceable buffers (pH 4.0, 7.0, 10.0) and results are recorded on pH meter calibration log sheet. Completed sheets are filed in the equipment notebook.

7.6 Incubators and water baths. All incubators and water bath temperatures are monitored twice daily. Water bath temperatures are recorded from four quadrants with an infrared laser thermometer and the average temperature is recorded on the log sheet. Incubator temperature is recorded from a fixed thermometer location for daily measurements. Uniformity of temperature is measured using an infrared laser thermometer aimed at all four corners of the incubator. Average temp. is only recorded if it deviates from the temperature displayed by the fixed thermometer.

8.0 Dishwashing. SOPs for washing of labware are available for reference at the dishwashing station. Only detergents designed for laboratory use are used for washing labware. All laboratory glassware is made of borosilicate glass and has readable measurement marks if applicable.

9.0 Glassware. All glassware is tested annually (or with a new lot number of detergent) for inhibitory residue by performing an inhibitory residue test in conjunction with the media performance tests required by a specific method. Inhibitory residue test results are recorded in the media performance log sheet. Completed media performance log sheets are filed in the media log notebook. All glassware are tested daily for acid or alkaline residues with a pH indicator and results are recorded on glassware log sheet. Completed glassware log sheets are filed in the media log notebook.

10.0 Sterility checks. All lot numbers of sterile materials that are purchased are subjected to a sterility test.

10.1 Sample Containers. A sample container is filled with sterile dilution water and the solution is filtered through a filter membrane (of a lot number previously tested for sterility). The filter membrane is placed on a sterile petri plate (of a lot number previously tested for sterility) containing plate count agar and incubated for 48 hours at 35 ^{o}C. Results are recorded on a media QC bench sheet. Completed bench sheets are filed in the media log notebook.

10.2 Dilution water. Sterile dilution water is purchased from an outside vendor and checked for sterility according to procedure outlined in section 10.1.

10.3 Membrane filters. One filter from each new lot is tested for sterility according to method outlined in section 10.1.

11.0 Analyst Demonstration of Capability. Each laboratory analyst shall have the required level of education and experience outlined by NELAC or individual method requirements, whichever is greater. Each new analyst must demonstrate an initial and at least annual ongoing demonstrations of capability. In addition, a new demonstration of capability must be performed each time there is a change in equipment, personnel, or test method. All associated supporting data (i.e. bench sheets) are retained in the PT notebook under the specified analytical method. Source Molecular Corporation does not use specialized work cells

11.1 Bacteria by membrane filtration. All bacteriological analysts are required to perform an initial proficiency (PT) sample prior to commencing laboratory analyses. PT samples are obtained from reputable NELAP-accredited vendor. PT samples are logged and processed according to all standard methods employed by the laboratory. Results**.** are logged in the PT notebook.

11.1.1 Total coliforms and *E coli* - Total coliforms and *E. coli* are analyzed by EPA Method 1604. The published method is available for reference in the laboratory.

11.1.2 Enterococci - Enterococci are analyzed by EPA Method 1600. The published method is available for reference in the laboratory.

11.1.3 Method Blanks and Sterility Blanks- A Method Blank is performed with each new media lot prepared for use. Sterility Blanks are performed each time the method is performed both prior to and after analysis and at least once every 10 samples analyzed. Filter funnels are rinsed three times with sterile rinse water between filtration series. Method Blank and Sterility Blank results and supporting documentation for bacterial analyses are filed in the Bacteriology PT notebook.

11.1.4 Analysis time. Membrane filtration is considered ended when more than 30 minutes has elapsed between successive filtrations.

11.1.5 Result Interpretation. At least once per month, the analysis in the 1 person laboratory must do repetitive counting on the same plate with no more than 5% difference between the counts. Results are recorded on laboratory bench sheet and filed in the bacteriology PT notebook.

11.2 *Cryptosporidium* and *Giardia* by EPA Method 1623. All parasitology analysts are required to perform an initial proficiency (PT) sample prior to commencing laboratory analyses. PT samples

are obtained quarterly from the United States Environmental Protection Agency as part of the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESTWR) Laboratory Accreditation Program. PT samples are logged and processed according to all standard methods employed by the laboratory. Results are logged in the PT notebook.

11.2.1 Matrix Spikes – Matrix spikes are required each time a new matrix is analyzed under EPA Method 1623. Matrix spike results and supporting documents are recorded in the OPR and Matrix Spike notebook

11.2.2 Ongoing Proficiency Testing - Ongoing Proficiency Testing is a method 1623 requirement and must be performed at least every week testing is being performed, or every 20 samples processed, whichever is greater. OPR results and supporting documentation are filed in the OPR and Matrix Spike notebook.

11.2.3 Method Blanks – Processing of a method blank is a method 1623 requirement and must be performed at least every week testing is being performed, or every 20 samples processed, whichever is greater. Method blank results and supporting documentation are filed in the OPR and Matrix Spike notebook.

11.3 Enteric Viruses by EPA, SM 9510, and ASTM D4994-89. All virology analysts are required to perform an initial proficiency (PT) sample prior to commencing laboratory analyses. PT samples generated in-house and are verified by the QC manager.

11.3.1 Matrix Spikes – Matrix spikes are performed each time a new matrix is analyzed. Matrix spike results and supporting documents are recorded in the OPR and Matrix Spike notebook

11.3.2 Ongoing Proficiency Testing - Ongoing Proficiency Testing is performed with each new lot of Beef Extract used in the method. OPR results and supporting documentation are filed in the OPR and Matrix Spike notebook.

11.3.3 Method Blanks – Processing of a method blank is performed with each new lot of Beef Extract used in the method. Method Blank results and supporting documentation are filed in the OPR and Matrix Spike notebook.

12.0 Quality Control. Positive and negative controls are run with each analysis to verify that filtration equipment and filters, sample containers, media, and reagents have not been contaminated by improper handling or preparation, inadequate sterilization, or environmental exposure. Each new batch of media prepared by the laboratory is tested for use with at least one pure culture of a positive control organism and at least one pure culture of a negative control organism. See Corporate Quality Control Manual for specific measures and procedures.

12.1 EPA Method 1603. Positive control – *E. coli*, Negative control – *Ent. faecium*

12.2 EPA Method 1600. Positive control – Ent. faecium, Negative control – E. coli

12.3 EPA Method 1623. Positive control - BTF Bio Easy Seed flow sorted *Cryptosporidium* and *Giardia* for matrix spikes and OPRs. Positive Staining control organisms are included with each staining kit.

12.4 Enteric Viruses by EPA ICR, ASTM D4994-89, SM9510. Positive Control – Poliovirus Lsc1, Negative Control – Sterile PBS. 40 Liters spiked water processed through entire method from filtration to cell culture.

13.0 Method evaluation and document control . The laboratory records and retains all microbiological validation data for as long as the pertinent test method is in force and for at least five years past the date of its last use.

14.0 Statistical calculations. Calculations, data reduction, and statistical interpretations are performed according to method requirements. See SOP Q-5.

15.0 Sampling

15.1 The laboratory has procedures for sampling on file in the SOP Notebook. Sampling plans are constructed on a per-project basis and are filed in the client file.

15.2 The sampling plan and procedures for sampling are taken to the locations where sampling is undertaken

15.3 Whenever reasonable, sampling plans are based on statistical methods

15.4 The sampling procedures address factors to be controlled to ensure validity of the environmental test results

15.5 The laboratory uses procedures dictated by individual analysis methods (when available) to obtain representative samples

15.6 All deviations, additions, or exclusions required by the client are recorded in detail with all appropriate sampling data, communicated to the client and appropriate personnel, and are included on final reports

15.7 Sampling procedures include steps to be taken to ensure adequate recording of pertinent data and operations relevant to sampling.

15.8 These records include sampling procedure used, identification of the sampler, environmental conditions, diagrams, sampling location, and statistical methods (if applicable) used.

15.8.1 See Field Data Sheet (FDS-1) located in sampling notebook

Version: 2
Revision: 2
Date accepted: 07/11/07 by Dr. T.M. Scott

16.0 EPA Method 1623 Equipment and Quality Control

All equipment and associated supplies for performance of EPA Method 1623 are available for use in the laboratory. All Quality Control checks and Equipment Maintenance records are on file in the appropriate notebooks.

16.1 Jouan 4 22 centrifuge (serial #49710072)

16.2 Immunomagnetic separation kits (Dynal), magnetic particle concentrators, and rotator

16.3 Primary staining antibodies (BTF – Easy Stain)

16.4 Olympus BX-51 TRF epifluorescence microscope with Differential Interference Contrast and excitation/band pass filters for FITC and DAPI (Serial # 2D10657)

16.5 Microscope adjustments (Hg bulb adjustments/replacement, transmitted bulb replacement, adjustment of oculars, calibration of ocular micrometer, establishing Kohler illumination) are performed according to manufacturer's instruction or EPA Method 1623. Checklist for microscope adjustments are on slide data sheets and are filed with supporting documentation. Bulb replacements are documented in Equipment Maintenance Notebook.

16.6 Acceptance Criteria. Acceptance criteria for Initial Demonstration of Capability (IDC), Ongoing proficiency (OPR), and Matrix Spikes (MS) are outlined in EPA Method 1623 and SOP Q-7

16.7 Analyst proficiency. See Quality Control section EPA Method 1623

16.8 Holding times. Dictated by Method and outlined in SOP P-1

17.0 Enteric Viruses by EPA ICR, ASTM D4994-89, SM9510 Equipment and Quality Control

17.1 Filters. 1-MDS filters are used for EPA ICR, 1-MDS or Filterite Filters are used for SM9510

17.2 Cell lines. Buffalo Green Monkey cell lines are used for all analyses. Only passage 117-250 are used for virus analysis. Other cell lines are also routinely used and specified in reports and data collection/analysis.

17.3 Cell culture positive control. Poliovirus –1 (attenuated) inoculated onto monolayer. Look for development of CPE within 48 hours (SOP V-3)

17.4 Cell culture negative control. Sterile PBS inoculated onto cell culture monolayer. No CPE observed (SOP V-3)

17.5 Cell culture maintenance. Each lot of calf serum and cell culture media is tested for sterility and performance and recorded in the media log notebook.

17.6 Methods manuals and SOPs for all test methods are available in laboratory SOP notebook and at work stations.

Version: 2
Revision: 2
Date accepted: 07/11/07 by Dr. T.M. Scott

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^{o}C for 1 min, 58^{o}C for 1 min, and 72^{o}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^{o}C for 10 minutes and 95^{o}C for 15 seconds followed by 40 cycles of 95^{o}C for 15 seconds and 57^{o}C for 1 minute. Gene marker copy numbers were calculated by reference to standard curves generated using synthetic target amplicons.

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.

Version: 2
Revision: 2
Date accepted: 07/11/07 by Dr. T.M. Scott