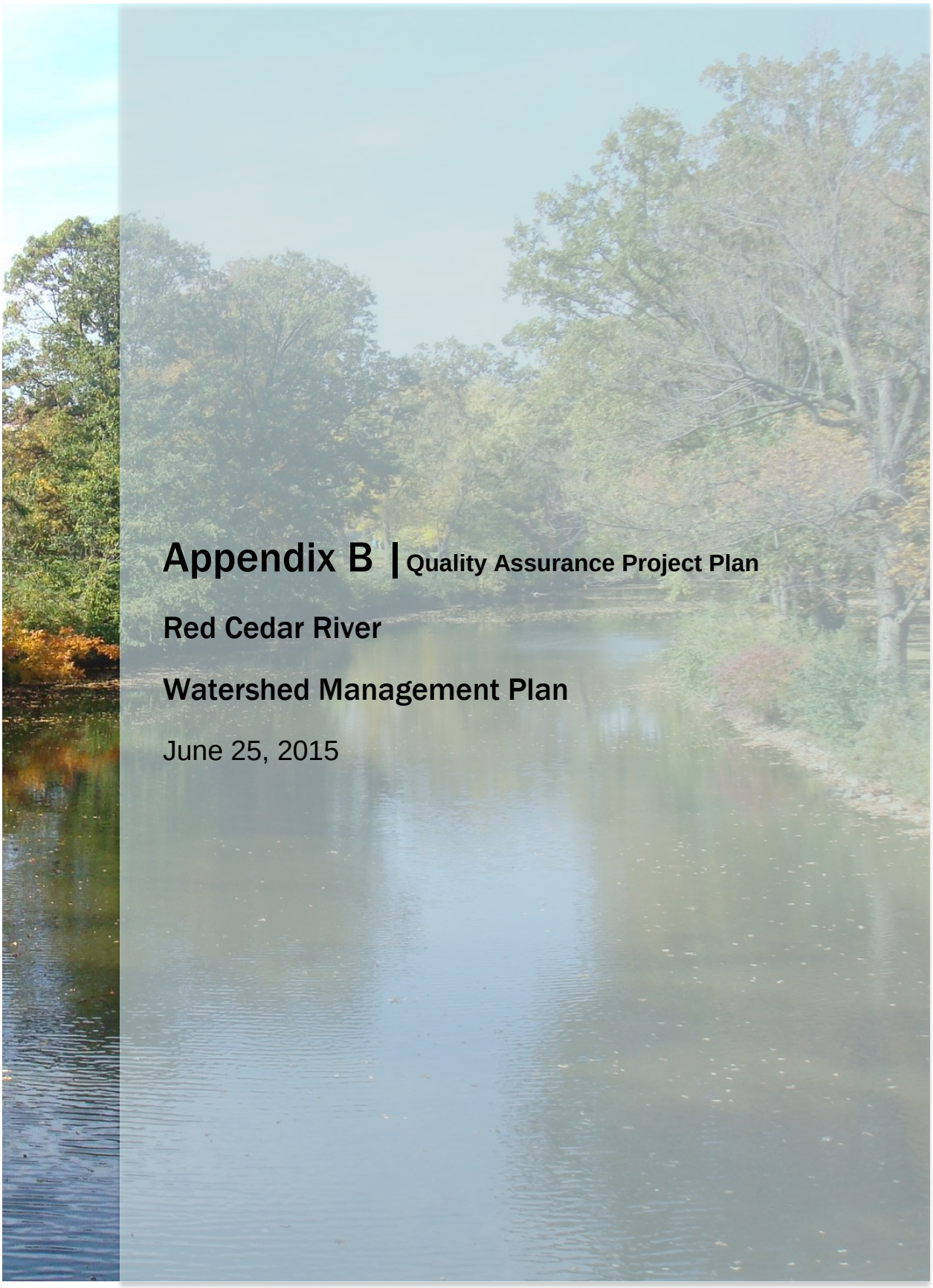

Appendix B | Quality Assurance Project Plan

Red Cedar River

Watershed Management Plan

June 25, 2015

Quality Assurance Project Plan (QAPP)
Red Cedar River Watershed Planning 2012 – 2014

Michigan Department of Environmental Quality tracking code: #2011-0014

August 21, 2012
Version 3

Ruth Kline-Robach 8-23-12

Ruth Kline-Robach, Grantee **Date**
Michigan State University-Institute of Water Research

Kalie Nye 8-23-12

Kalie Nye, Graduate Student **Date**
Michigan State University

8-23-12

Joe Rathbun, **Date**
Nonpoint Source Monitoring Coordinator
Michigan Department of Environmental Quality

for Robert Day, 8/23/2012

Robert Day, **Date**
Nonpoint Source Unit Chief
Michigan Department of Environmental Quality

Quality Assurance Project Plan (QAPP)
Red Cedar River Watershed Planning 2012 – 2014

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Ruth Kline-Robach, Grantee Date
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Nonpoint Source Monitoring Coordinator
Michigan Department of Environmental Quality

Robert Day, Date
Nonpoint Source Unit Chief
Michigan Department of Environmental Quality

Table of Contents

QAPP Organization and Project Description

Distribution List

Michigan State University

- Ruth Kline-Robach
- Kalie Nye

Michigan Department of Environmental Quality (MDEQ)

- Joe Rathbun, Nonpoint Source Monitoring Coordinator
- Susan Doty, Project Manager
- Thad Cleary, Grand Administrator
- Molly Rippke, Aquatic Biologist

Streamside Ecological Services

- Aaron Snell

Tri- County Regional Planning Commission

- Erin Campbell

Project Organization

The Institute of Water Research at Michigan State University is responsible for grant administrative activities. Together, Michigan State University- Institute of Water Research (MSU-IWR), Streamside Ecological Services (SES), and Tri-County Regional Planning Commission (TCRPC) (the internal project team) will collect and analyze data for use in developing a Watershed Management Plan. Throughout the process, the internal project team will collaborate with the Ingham County Health Department (ICHD) and the Michigan Department of Environmental Quality (MDEQ) project contacts.

Table 1. Red Cedar River Watershed Planning Project Leadership- Internal Project Team

Name and Title	Responsibilities	Contact Information
Ruth Kline-Robach, Outreach Specialist, Michigan State University-Institute of Water Research	Project Coordinator, data analysis	kliner@msu.edu (517) 355-0224
Kalie Nye, Graduate Assistant, Michigan State University	QAPP Development, *E. coli* sampling	nyemicha@msu.edu 517-353-2244
Aaron Snell, Aquatic/Restoration Biologist, Streamside Ecological Services	Technical expertise and data analysis	snell@streamsideeco.com (616) 238-7372
Erin Campbell, Assistant Environmental Planner, Tri-County Regional Planning Commission	Data analysis	ecampbell@mitcrpc.org 517.393.0344 ext. 22

Project Description

Sample data collected under this QAPP will be used in the Red Cedar River Watershed Planning Project. The intent of the Red Cedar River Watershed Planning Project is to develop a plan to restore and protect water quality. To make this task most manageable, the watershed management plan and thus this QAPP will focus on four to eight 12-digit hydrologic unit code (HUC) subwatersheds of the Red Cedar River Watershed.

The Red Cedar River watershed (HUC 04050004) is approximately 294,496 acres (461 sq. miles). The headwaters are located in eastern Livingston County, in the south-central portion of the Lower Peninsula; the river flows in a westerly direction into and across Ingham County. The river meets the Grand River near the Ingham and Eaton County border in downtown Lansing. Land use in the watershed area is as follows: 59% agriculture/bare; 14% residential/commercial/industrial (intensity developed); 13% forest/range; 14% wetland/water.

Portions of the watershed are included in a Draft Total Maximum Daily Loads (TMDL) for *Escherichia coli* (*E. coli*). Portions of the watershed are anticipated to be included in dissolved oxygen (DO), mercury, and polychlorinated biphenyl (PCB) TMDLs.

The Wolf Creek, Dietz Creek, Doan Creek, Squaw Creek, Coon Creek, Headwaters Sycamore Creek, Sycamore Creek, and Red Cedar subwatersheds have *E. coli* TMDLs under development (subwatershed naming from National Hydrography Dataset). TMDLs to address low DO levels are also scheduled for 2012 and 2017 in the Red Cedar River, Mud Creek, Sycamore Creek, Cook and Thorburn Drain from Cedar Lake Upstream, and the Headwaters Sycamore Creek subwatersheds.

The emphasis of this watershed planning effort and this QAPP will be focused on collecting data to address the *E. coli* TMDL and *E. coli* water quality standard (WQS) exceedances in the Red Cedar Watershed. Sampling locations will be selected based on results of previous studies done on the watershed and in areas where there are data gaps. Data sources used in selecting sampling sites include, areas determined to benefit from additional sampling as outlined in the *Total Maximum Daily Load for E. coli in portions of the Red Cedar River and Grand River Watersheds; including Sycamore, Sullivan, Squaw, and Doan Creeks: Ingham, Eaton, Clinton, Jackson, and Livingston Counties* report (MDEQ, 2012), priority areas determined in the 2001 watershed planning work, areas identified as benefiting from additional sampling through *E. coli* transport modeling, and areas of high sedimentation as determined by High Impact Targeting (HIT) modeling results.

Sampling is expected to take place over two sampling seasons. Thirteen sites will be sampled for *E. coli* in the late summer and early fall of 2012. Data collected from this sampling effort will be used in developing an expanded monitoring plan for spring and summer of 2013. The expanded monitoring plan is expected to include additional parameters and different sampling sites. A QAPP addendum or new QAPP will be submitted for that effort.

The Red Cedar Watershed Management Plan is meant to complement the existing stormwater management plan developed by the Greater Lansing Regional Committee for Stormwater Management (GLRC) for the urbanized areas and their municipal separate storm sewer system (MS4) permit requirements. In addition, data will be shared with the Middle Grand River watershed planning project.

Training Requirements/Certification

Persons responsible for sampling *E. coli* under this project and QAPP will have prior *E. coli* sampling experience or will be trained by others with experience in *E. coli* sampling.

Measurement/Data Acquisition

Study Objectives

The objective of the watershed sampling effort is to collect supplemental data to better understand pollutant conditions, sources, and causes. The objective of the 2012 sampling effort is to collect additional *E. coli* data in order to finalize four to eight priority subwatersheds and to identify those tributaries that are contributing higher levels of *E. coli.*

Currently, in most of the subwatersheds listed as impaired, the *E. coli* TMDL reaches extend throughout the majority of the subwatershed. However, the TMDL locations were identified primarily from monitoring data taken from the main branch of the Red Cedar River. This study will assess the *E. coli* concentrations at various locations within certain tributaries of the Red Cedar River.

Study Design Description

Monitoring Location Selection

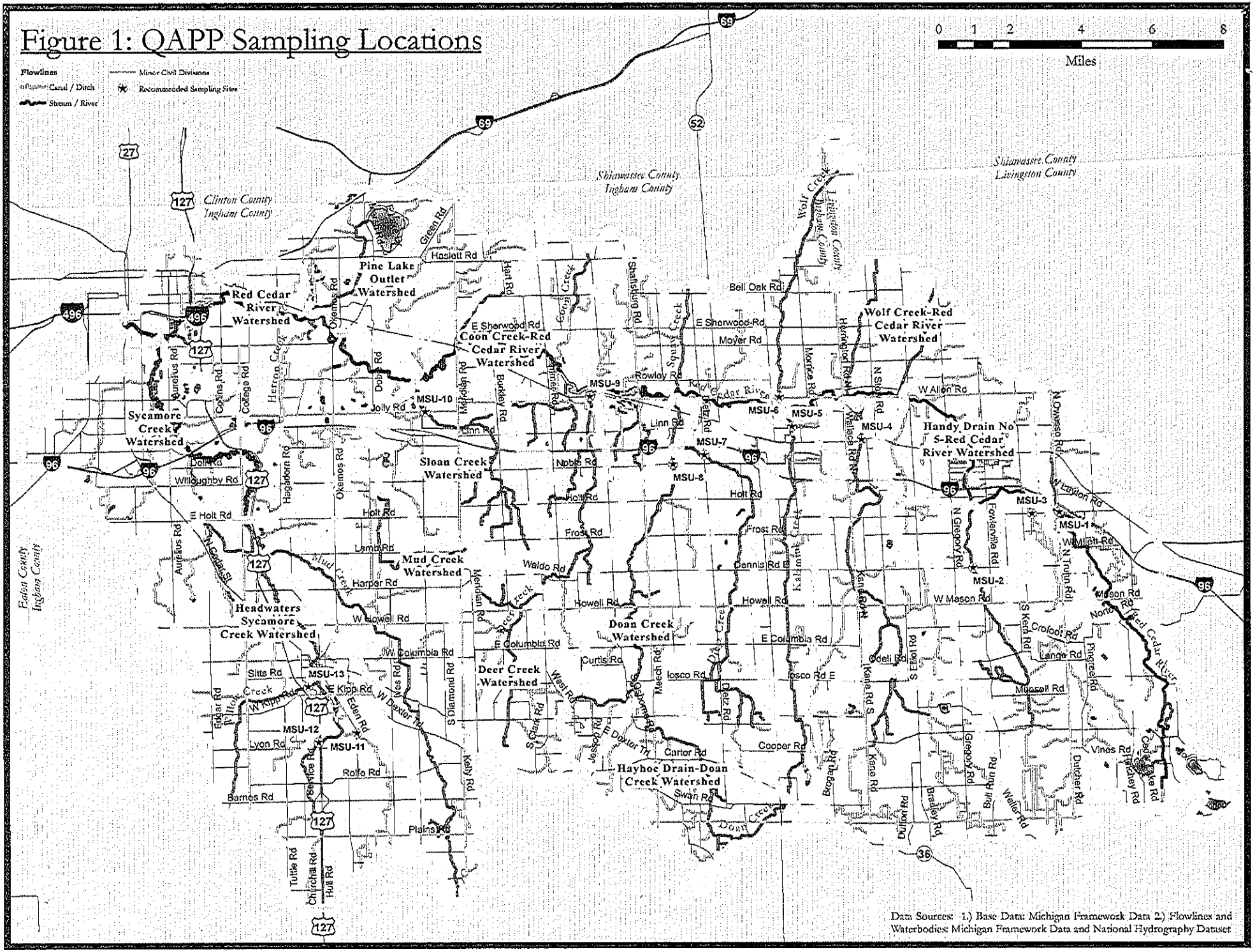
An assortment of existing data has been compiled and reviewed. Sources of that data include past and current monitoring studies, information provided by watershed stakeholders and academicians, and historical watershed planning documents. Additional monitoring sites were selected by reviewing previous studies and by running a HIT sediment model for the watershed. Recommendations from the report entitled *Total Maximum Daily Load for E. coli in Portions of the Red Cedar River and Grand River Watersheds; including Sycamore, Sullivan, Squaw, and Doan Creeks: Ingham, Eaton, Clinton, Jackson, and Livingston Counties* (MDEQ, 2012) and its author Molly Rippke (personal communication, July, 20, 2012) were also considered. Priority areas determined in watershed inventory work conducted in 2001, *E. coli* transport modeling conducted by faculty members at Michigan State University, and through HIT modeling conducted at the MSU-IWR were also incorporated.

The various studies and data sources prioritized the subwatersheds differently, and four to eight priority subwatersheds are not apparent across the various data sources. In addition, there are obvious gaps in the available sampling data. This study will address those gaps by collecting data at locations that have not yet been sampled. In addition, sites prioritized for additional monitoring by Molly Rippke (personal communication, July, 20, 2012) will be included in the 2012 sampling regime. Where possible, the sampling locations will be at road and stream crossings with right-of-way access.

A map of the watershed showing proposed monitoring stations is included as Figure 1.

The study will focus initially on *E. coli* to coincide with the *E. coli* TMDL that was recently written for portions of the watershed. In addition, because of the unusually dry summer season,

Figure 1: QAPP Sampling Locations



temperature and depth of water measurements will be recorded during one sampling event at each sampling location with the intention of correlating *E. coli* results with those parameters and to better compare the 2012 data with the historical data.

Subwatersheds in which *E. coli* samples will be collected include:

- Coon Creek
- Dietz Creek
- Doan Creek
- Handy Drain No. 5
- Handy Howell Drain
- Headwaters Sycamore Creek
- Kalamink Creek
- Middle Branch
- Sloan Creek
- West Branch
- Wolf Creek

Data collected during this sampling effort will be combined with the other available data and reports in order to prioritize four to eight subwatersheds. It is anticipated that the 2013 sampling will focus on potential sources and causes of the impairments in the priority subwatersheds, and will include additional parameters to better understand pollutants and potential sources in the priority subwatersheds.

Sampling Process

Thirteen sites will be sampled weekly for four weeks beginning the week of August 27, 2012. Samples will be collected weekly on Wednesday and Thursday each week. In the event of unexpected challenges, data will be collected as close as possible to the originally scheduled sampling date.

At each *E.coli* sampling location, three samples (left, right, and center) will be collected. The three results will be used to calculate a geometric mean for each sampling location for each week. Temperature and depth of water will be measured and recorded after the *E. coli* samples have been collected each week.

To more easily manage sample data, sample stations will have a unique name. Samples sites will be labeled "MSU-#", and the # will be replaced with their unique sample site number beginning with 1 and increasing numerically. Samples will be numbered and labeled with their own unique sample ID number. The unique name will allow differentiation between other data sets and between sample sites.

Weather conditions on the dates of the sampling events will be recorded using data posted on the Michigan State University Enviro-Weather website East Lansing station (http://www.enviroweather.msu.edu/weather.php?stn=msu). In addition, rainfall amounts in the 48 hours preceding the sampling will be recorded.

If problems with sampling site access arise, a new sampling site will be selected in close proximity to the originally planned site. If field conditions warrant a large deviation from the sampling proposed in this QAPP, we will contact our MDEQ project manager to discuss the changes.

If insufficient water is available at a site to allow for sampling, a site nearby the originally planned site will be used for sample collection. Or if possible, the sediment at the bottom of the stream will be manually dredged, the suspended solids allowed to settle, and water allowed to flow from upstream into the area before the sample is collected.

Data Quality Objectives

Obtaining quality and useful data is necessary to most effectively inform the watershed management planning process. Table 2 below describes measures that will be taken to ensure that data is accurate, precise, comparable to other studies, and representative of field conditions.

Table 2. Red Cedar River Watershed Management Plan Data Quality Objectives

Activity	Accuracy	Precision	Representativeness /Comparability
E.coli sampling	Collect a blank every 20th sample or once per survey, whichever is more frequent Measured value within 25% of known standard concentration Field blanks with a bacteria count of ≤ 10 CFU	Collect a field duplicate every 20th sample or once per survey, whichever is more frequent Relative Percent Difference (RPD) of ≤ 25% Duplicate samples exceeding the 25% RPD but still within the same regulatory category will be considered acceptable	Three samples (right, left, center) for each sampling location to generate a geometric mean Use standard sampling and analysis methods
Temperature	Follow Field Protocol included in QAPP Instrument rated for ±1.0 °C	Collect a field duplicate every 20th sample or once per survey, whichever is more frequent Relative Percent Difference (RPD) of ≤ 20%	Same as accuracy
Water Depth	Follow Field Protocol included in QAPP	Same as accuracy	Same as accuracy

The completeness data quality objective (DQO) will be attained by collecting each proposed sample at each of the proposed sampling sites and event times. The dataset will be considered complete if 90% of the data is useable and meets all DQOs. Any problems that arise in sampling will be handled as described in the Sampling Process section above.

Sample/Data Collection and Analysis Procedures

Industry accepted collection and analysis procedures will be followed and are described in detail below. Table 3 below lists the analytical method, detection limit, sample volume, bottle type, preservative, and hold time that will be used for this sampling effort.

Table3. Red Cedar River Watershed Management Plan Sample Collection and Analysis Details

Parameter	Method	Detection Limit	Sample Volume (mL)	Bottle Type	Preserva-tive	Hold Time
E. coli	EPA 1103.1	Lower: 10-10,000 CFU /100 mL OR Upper: 10-1,000,000 CFU /100 mL	100	Plastic Unit 30	Sodium Thiosulfate	6 hours
Temperature	Cole Parmer Remote Probe Thermometer	± 0.1 °C	Not applicable, measured with a field instrument.			
Water Depth	Meter Stick Manual Measurement	1 mm	Not applicable, measured with a field instrument.			

E. Coli

Samplers will follow the procedures described in the MDEQ Drinking Water Laboratory EQP 2300 form included in the Appendix. *E. coli* samples will be collected away from the banks and in the stream current. Sampling locations will be chosen that are representative of average stream conditions to be sampled. The laboratory form will be labeled with the date, time, sampler initials, and location-specific sample identification. The bottle will be labeled with the unique sample ID.

Samples will be collected by wading into the stream, from the banks of the stream where the stream is narrow, or from a bridge above the stream. By approaching the sampling site from

downstream, walking with caution, and/or by sampling from the banks of the stream, the bottom of the stream will be disturbed as little as possible to avoid including any disturbed sediment from the bottom. If the sediment is disturbed the sampler will wait until the sediment settles to collect the samples. The sampler will face upstream while collecting samples. To collect the sample, the sampler will plunge the bottle into the water with the top facing downward while holding the bottom of the bottle. The bottle will be pushed away from the sampler and upstream. A one inch air gap below the lid will be left, and the cap will be placed on the bottle while avoiding contamination into the cap or bottle from other sources. If the stream is too shallow to plunge the bottle into the water facing downward, the bottle will be pushed into the water on its side, facing downstream until submerged, and turned to face upstream to collect the sample. An extension pole may be used to collect samples in deep water or to collect samples while the sampler stands on the banks of the stream. The sampler will collect the water sample eight to 12 inches below the water's surface or, where the stream is shallow, half-way between the water surface and the bottom of the stream. (United States Environmental Protection Agency Office of Water, 1997). When sampling from the bridge, the sampler will tie a string or rope to the top of the bottle, unscrew the cap carefully and set it aside so that the inside of the cap remains untouched. The sampler will lower the bottle using the rope, bottom first, to the water by gravity. The bottle will be submerged into the stream using stream water to weight and lower the bottle to the appropriate stream depth. Once the bottle is filled, it will be pulled out of the water and up to the bridge. A one inch air gap below the lid will be left, and the cap will be placed on the bottle while avoiding contamination from getting into the cap or bottle from other sources. The bottle will be labeled and placed in the cooler. GPS coordinates and the nearest intersection will be identified and recorded for each sampling location on the field form.

A laboratory supplied EQP 2300 form will be filled out by the sampler or sampling team. Samples will be delivered to the MDEQ Drinking Water Analysis Laboratory at 3350 North M.L. King Blvd., P.O. Box 30270, Lansing, MI 48909, within 6 hours of being collected and between the hours of 8 a.m. to 3 p.m. on Monday - Thursday. The laboratory can be reached at 517-335-8184. Once delivered to MDEQ lab, MDEQ assumes responsibility for the samples and is to follow all approved laboratory practices for sample handling.

During the first week of sampling, samples will be analyzed for the presence of *E. coli* colony forming units (CFUs) in the 10-1,000,000 CFU range using the NPEC_High test method by membrane filtration. Samples that have results from the first week of sampling above 7,500 CFU (25% of 10,000 CFU) will continue to be tested using the NPEC_High method for the remainder of the four week sampling event. Samples that have results below 7,500 CFU will be sampled using the NPEC_Low method for the remainder of the four week sampling event. In the event that sample results from the first week of sampling have not yet been reported by the laboratory before the second week of sampling, the NPEC_High method will be used again

for the second week of sampling. In that case, the sampling methods for the third and final weeks of sampling will then be determined by the first and second weeks sampling results using 7,500 CFU as the level triggering the use of the NPEC_High method.

Depth of Water

Depth of water will be collected using a meter stick each week of sampling. Water depth will be measured in the center of the stream at each sampling site. The depth will be measured from the top of the sediment in the bottom of the stream to the water surface and recorded to the nearest centimeter on the field form. The stream flow (high, medium, low) will also be recorded using observations of the stream conditions and any signs of previous water lines for comparison. This measurement may also be taken from the bridge. To measure the depth to water from the bridge, the "tape-down" method will be followed. A measuring tape with a weight on the end will be lowered to the water. The measurement to the bottom of the stream will be measured in relation to a spot on the bridge. The measurement to the surface of the stream will also be measured in relation to the same spot on the bridge and the difference will be calculated and recorded as the depth of water.

Temperature

Temperature will be measured using a Cole Parmer Remote Probe Thermometer and recorded to the nearest tenth of a degree Celsius each week of sampling. Temperature will be measured from near the same location and depth in the stream as where the center *E. coli* sample was collected at each sampling site. The time and temperature will be recorded on the field form. This measurement may also be taken from the bridge. If the temperature is taken from the bridge, after *E. coli* samples have been taken, a rope and bucket will be used to collect water from the stream. The temperature of the water in the bucket will be taken immediately following collection and recorded as the stream temperature.

Any additional notable observations of the stream at each sampling site may also be recorded on the field form.

Quality Control Requirements

Measures will be taken to obtain data of an acceptable quality.

One field blank will be collected for every twenty *E. coli* samples collected or once per trip, whichever is more frequent. Field blanks will be standard *E. coli* sample bottles filled with

sterilized water and labeled to indicate they are a field blank (United States Environmental Protection Agency Office of Water, 1997).

In addition, one field duplicate sample will be collected for every twenty *E. coli* samples, or one field duplicate per trip, whichever is more frequent. To fill duplicate samples, a second *E. coli* sample bottle will be filled at the same sample location and time as another *E. coli* sample. The sample will be labeled to indicate it is a duplicate (United States Environmental Protection Agency Office of Water, 1997). Results of field blank and field duplicates tests will be included with the rest of the sample data results.

A field duplicate measurement of temperature will also be collected for every twenty temperature readings, or once per trip, whichever is more frequent. To record a duplicate temperature field measurement, the temperature probe must be removed from the water and allowed to regulate its temperature between temperature readings.

Laboratory quality control measures for *E. coli* samples are detailed in SOP#603 in the appendix. Some of the laboratory quality control measures include method blank control, negative daily control, positive daily control, and initial demonstration of capability. In addition, ongoing precision and recovery measures are taken.

In addition, the person responsible for sampling and data management and/or the project team will:

- Document any changes to the proposed sample collection and analysis procedures;
- Ensure supplies are inspected prior to use;
- Verify the appropriate laboratory analytical procedures and quality control procedures were followed, and take corrective action if necessary; and
- Ensure all data are reviewed, recorded, and archived.

Data Analysis and Interpretation

E. coli sample data collected will be reviewed and compared against historical data, across sampling sites, and against the respective WQS. The daily maximum standards for Total Body Contact (TBC) and Partial Body Contact (PBC), 300 CFU/100mL and 1,000 CFU/100mL respectively, will be the WQS used in this data analysis. The geometric mean of each sampling site on each date will be calculated. Data trends, areas with frequent WQS exceedances, and areas of infrequent WQS exceedances will be of interest during the data interpretation.

The results will be interpreted by the internal project team in consultation with the MDEQ project contacts.

Geometric mean results calculated from the sampling data will be used to rank and prioritize the sites relative to each other and relative to existing data. Sites exceeding both the TBC and PBC standards will be the top priority and will warrant additional investigation and/or monitoring in the area at a later time.

Weather, temperature, and depth of water measurements will be used to help understand *E. coli* results based on known *E. coli* behaviors.

Instrument/Equipment Calibration, Testing, Inspection and Maintenance

Laboratory calibration standards for *E. coli* samples are detailed in SOP#603 in the appendix.

It is assumed that the laboratory meets testing, inspection, and maintenance requirements as it is an MDEQ laboratory.

Supplies Inspection

Samplers will verify laboratory bottles are properly sealed and include the appropriate preservative prior to use.

Data Acquisition Activities not covered by this QAPP

Historical and existing data have been and will be collected for use in the Watershed Management Planning process. Data will be collected from reputable and reliable sources.

GIS data is planned to be collected from TCRPC, Michigan Geographic Data Library (http://www.mcgi.state.mi.us/mgdl/), MDEQ, the National Hydrography Dataset, and the U.S. Department of Agriculture – Natural Resources Conservation Service.

E. coli, macroinvertebrate, dissolved oxygen, and other applicable chemical and biological sample data will be obtained from other studies and organizations where applicable data may be available. Organizations contributing to the data that will be used in this Watershed Management Planning Process include: MDEQ, Delhi Township, Ingham County Health Department, Eaton County Conservation District, Charter Township of Meridian (Lake Lansing Special Assessment Advisory), Ingham County Drain Commissioner, Livingston County Drain Commissioner, and Livingston County Health Department.

Ingham County Health Department and MDEQ data were used in the development of the TMDL by MDEQ so are assumed to be reliable sources of data. Eaton County Conservation District and Delhi Township have an MDEQ approved QAPP. Data collected through work done by the Ingham County Drain Commissioner, Livingston County Drain Commissioner, and Livingston County Health Department are assumed to be reliable as work was completed with guidance

from MDEQ. MDEQ is reported to have participated in the project involving data collection for the Township of Meridian (Lake Lansing Special Assessment Advisory) so it is also assumed to be reliable (Progressive AE, 2002).

Data Validation and Reporting

Data Review, Validation, and Verification

The internal project team will review the data at the completion of the four week sampling process.

A review will be conducted to ensure the data collected is complete. Completed EQP 2300 forms and field forms will be reviewed to verify that they were thoroughly filled out. All data collected will be compared against that planned in this QAPP for completeness and methods. *E. coli* data will be reviewed for each set of laboratory results received for reasonableness.

Field blank sample laboratory results will be reviewed to ensure an unacceptable amount of bacteria counts were not recorded in the field blank indicating sampling or equipment errors. (United States Environmental Protection Agency Office of Water, 1997). Bacteria counts in field blanks should be less than or equal to 10 CFUs. An RPD calculation will also be done to verify the *E. coli* CFU amounts are within 25% of known standard amounts used in laboratory spiked samples.

Field duplicate temperature measurement results will be reviewed to ensure precision in field measurements. An RPD calculation will be done to verify this and 20% will be used as an acceptable range.

Field duplicate sample laboratory results will be reviewed to check sampling and laboratory analysis precision. Field duplicate samples should have *E. coli* counts comparable to the original sample *E. coli* counts taken at the same location and time per 100 mL (United States Environmental Protection Agency Office of Water, 1997). An RPD calculation will be done to verify this and 25% will be used as the acceptable range. If field duplicate samples are outside of the acceptable 25% RPD range, but still within the same regulatory category, both exceeding 1,000 CFU/100 mL or both between 300 CFU and 1,000 CFU/mL, the sample data will be considered valid and useful for the purpose of this watershed management planning process.

Any quality control data completed and provided in the results by the laboratory will be reviewed. In the event of a discrepancy, the laboratory or sampler will be contacted to clarify

any observed errors. Additional data collection may be required to be collected to fill in any data gaps.

Reconciliation of Data with DQOs

Data deemed acceptable after the review, validation, and verification will be approved as having met the accuracy, precision, comparability, representativeness, and completeness DQOs of the sampling event covered under this QAPP. Data that does not meet these requirements will be discussed in the report developed upon completion of the sampling events.

Additional sampling locations and/or parameters will be considered for the 2013 sampling season in the report developed upon completion of the sampling events.

Data Management

Field forms and laboratory analysis result copies will be stored in a file folder at MSU-IWR. Data will be compiled into a spreadsheet using the United States Environmental Protection Agency STORET chemical data spreadsheet as a template (available from http://www.michigan.gov/deq/0,1607,7-135-3313_3682_3714-152031--,00.html, accessed July 25, 2012). Data will be backed up onto an external drive. Data will be stored as a complete set and will be in one location at the MSU-IWR, stored for five years minimum.

Data Reporting

Data results will be compiled by the internal project team. The results will be compiled into a report at the end of the four week sampling event and submitted to the MDEQ project manager and MDEQ Project Administrator with the project quarterly report.

References

Michigan Department of Environmental Quality Water Resources Division. (July 2012). *Total Maximum Daily Load for E. coli in portions of the Red Cedar River and Grand River Watersheds; including Sycamore, Sullivan, Squaw, and Doan Creeks: Ingham, Eaton, Clinton, Jackson, and Livingston Counties.*

Progressive AE. (March, 2002). *Management Plan for Lake Lansing and Its Watershed.*

United States Environmental Protection Agency Office of Water. (November, 2007). *Volunteer Stream Monitoring: A Methods Manual.* (EPA 841-B-97-003).

Appendix

Standard Operating Procedures

Michigan Department of Environmental Quality Drinking Water Laboratory EQP 2300

Michigan Department of Environmental Quality SOP #603 Escherichia Coli in Water by Membrane Filtration (NPEC)

Field Form

Red Cedar Monitoring Data

BILLING INFORMATION *PLEASE PRINT*

Name	DWL Account Number
Mailing Address	
City / State / Zip	
Check#, if applicable (pay to State of Michigan)	Amount enclosed

DEQ DRINKING WATER LABORATORY - LANSING
DEPARTMENT OF ENVIRONMENTAL QUALITY

REQUEST FOR WATER ANALYSIS

- **FEE AMOUNTS ON THIS FORM ARE EFFECTIVE JAN. 1, 2010.** Fee amounts are subject to annual changes.
- Sample Collection Instructions are on the back of this form
- **PREPAYMENT OR *APPROVED* DWL CREDIT ACCOUNT NUMBER IS REQUIRED FOR TESTING.**

WSSN (Type I-II Public Water) or Pool Serial Number	Does sample contain chlorine? ☐ Yes ☐ No	**DO NOT SEND CASH!**

SAMPLE SOURCE - *Circle One*
- 0 - Single Family Dwelling
- 1 - TYPE I (community, apartment, subdivision, mobile home park, etc., with 25 or more residents year round)
- 2 - TYPE II (school, industry, restaurant, office, etc., serving 25 or more persons - 60 days or more per year)
- 3 - TYPE III (all other public supplies, duplex, small office, etc.)
- 7 - Surface Water (includes bathing beach and wastewater discharge)
- 8 - Swimming pool or Spa
- 9 - Other

SAMPLING PURPOSE - *Circle One*
- 0 - Routine Monitoring
- 1 - Real Estate Transaction
- 2 - Repeat Sample
- 3 - Repair/Construction/New Well
- 5 - Water Quality Problem
- 9 - Other

SAMPLE POINT - *Circle One*
- 1- Public System Well
- 2- Public System Surface Water
- 3- Untreated Public Distribution System
- 4- Treated Public Distribution System
- 5 - Untreated Private Well
- 6 - Treated/Softened Private Well
- 7 - Pressure Tank/Plant Tap
- 9 - Other

SENDER INFORMATION *PLEASE PRINT* NOTE: RESULTS WILL BE AUTOMATICALLY COPIED TO LOCAL COUNTY HEALTH DEPARTMENT

Name	E-mail address
Mailing Address	Area Code & Phone number
City / State / ZIP Code	

SAMPLE COLLECTION INFORMATION *PLEASE PRINT*

Sample Collector Name	Date Collected	Time Collected — Circle One AM PM

☐ Do **NOT** analyze my sample(s) if received past the EPA specified hold time. (Hold times are indicated on the sample bottle.) **THE DATE AND TIME COLLECTED MUST BE FILLED OUT!**

☐ Analyze my sample(s) even if received beyond the EPA specified hold time.

NOTE: If a selection is not made, your sample(s) will be analyzed. Although samples analyzed beyond hold time typically cannot be used for compliance purposes, the results may still have informational value.

Collector Code *Circle One* — 0 - County Personnel 1- Water Supply Operator 2- DEQ DW staff 3- Private Citizen 4 - DEQ Staff other than DW 6- MDA Staff 9- Other	System/Owner Name	
Collection Site (Street Address)	Township (if known)	Section (if known)
City / County	ZIP Code	Well (if more than one) Number
Sampling Point (kitchen, bath, etc.)	Site Code or Permit Number (if known)	

TESTING REQUEST INFORMATION (REQUIRED)

TEST CODE	REQUIRED UNIT #	DRINKING WATER OR POOL/SPA TEST	FEE	☑
B	30	Water Coliforms (Bacteriology) *30 hour hold time*	$16.00	
R	32	Automated Partial Chemistry, *including Fluoride, Chloride, Hardness, Nitrate, Nitrite, Sulfate, Sodium and Iron*	$18.00	
CAS	36ME	Arsenic	$18.00	
CPB	36ME	Lead	$18.00	
CCUB	36CC	Lead/Copper for corrosion control	$26.00	
CXVO	36VO	Volatile Organic Compounds	$100.00	
TEST CODE	**REQUIRED UNIT #**	**SURFACE OR WASTEWATER TEST (Pond, Lake, Ditch, etc.)**	**FEE**	
NPEC-LO	30	E. coli (Counts 10 - 10,000) *delivery to lab-6 hours*	$15.00	
NPEC-HI	30	E. coli (Counts 10 - 1,000,000) *delivery to lab-6 hours*	$25.00	
NPFC-LO	30	Fecal Coliform (Counts 10 - 10,000) *delivery to lab-6 hours*	$15.00	
NPFC-HI	30	Fecal Coliform (Counts 10 - 1,000,000) *delivery to lab-6 hours*	$25.00	

INSTRUCTIONS:
- Check box next to Test Code(s) of desired analysis.
- Check the UNIT# on bottle to ensure you have the REQUIRED UNIT for desired analysis.
- For other types of testing, enter the TEST CODE, UNIT# (located on the sample bottle) and FEE in the area on the right side of this section.
- Refer to the full Testing Fee Schedule available from county health departments and DEQ Drinking Water Laboratory for other types of testing. **Fee amounts are subject to annual changes.**

TEST CODE	UNIT#	FEE
TOTAL OF ALL FEES »		

EQP 2300 (Front) 12/2011)

Submit White Copy With Sample - Keep Yellow Copy For Your Records

- A form is required for **each sample site** (Collection Site, Sampling Point, and Date/Time must be the same for all samples with this form).
- **Complete all parts of this form which apply.** Samples not properly identified or not having clear test requests MAY NOT be tested.
- Fill in your email address, if you would like a copy of the report emailed when completed.
- **For additional information contact your local county health department or the Drinking Water Laboratory, (517) 335-8184 or visit our web site: http://www.michigan.gov/deq**

Allow two weeks for results on most testing.

SAMPLE COLLECTION INSTRUCTIONS

UNIT#	INSTRUCTIONS
30	1. **This testing unit contains preservatives in the sample bottle.** Do not rinse the bottle with sample. Do not open the bottle until ready to collect the sample. Do not touch the inside of cap or bottle. 2. If not collecting sample from a tap (lake, pool, etc.), plunge bottle mouth down, move in continuous arc down and back up from water, discard top half-inch or to 100 ml line. 3. If using a sample tap, select a **clean** (disinfect as necessary) faucet and remove such attachments as aerators, dishwasher connectors, etc. Allow water to run for about ten minutes at full flow from the sampling tap. Reduce flow to avoid splashing, and collect the sample directly into the bottle. Do not use an intermediate container. Do not allow water from the outside surface of the faucet to drip into the bottle. **Fill bottle only to the bottom of neck, or to 100 ml line.** 4. Most bacteriological testing has a 30 hour EPA hold time. Samples must be received at the laboratory before the hold time expires. Surface water samples must be received at the laboratory within 6 hours of sampling.
32*, 33* **36AC** **36CN, 36HA*** **36HB, 36LP** **36ME, 36PT**	1. **Sample bottle may contain preservative** (refer to unit label on bottle). Do not rinse bottle with sample. Do not open the bottle until ready to collect the sample. Do not touch the inside of cap or bottle. 2. Select a clean faucet and remove such attachments as aerators, dishwasher connectors, etc. Allow water to run for about ten minutes at full flow from the sampling tap. Reduce flow to avoid splashing, and collect the sample directly into the bottle. Do not use an intermediate container. Do not allow water from the outside surface of the faucet to drip into the bottle. **Fill bottle to the bottom of neck.**
36TO* **36VO*** **36VO-NP***	1. **The sample vials contain preservative. Tap each vial in upright position to drain preservatives from cap. Do not rinse vial before collection.** 2. Do not open the vial until ready to collect the sample. Do not touch the inside of cap or vial. Select a clean faucet without attachments or leaking stem. Allow water to run for ten minutes at full flow. 3. Reduce flow and collect the sample directly into **all** vials provided. a. **For 36TO,** fill vial until water rounds at the top of vial. b. **For 36VO,** fill vial HALFWAY. Add 2-3 drops of the provided acid from small dropper bottle. Completely fill vial until water rounds at the top of vial. 4. **Cap and invert to check for air in vial.** THE SEPTA (RUBBER PART INSIDE CAP RING) MUST BE SMOOTH SIDE DOWN IN CONTACT WITH SAMPLE TO AVOID POSSIBLE CONTAMINATION. 5. If air is observed in inverted sample, remove cap, add water **(DON'T DUMP SAMPLE)** and recap as instructed.
36CNa	1. **Enclosed vial contains dilute preservative and caution should be exercised. This testing unit also contains preservatives in the sample bottle. Tap unit in upright position to drain preservatives from cap. Do not rinse bottle before collection.** 2. Do not open the bottle until ready to collect the sample. Do not touch the inside of cap or bottle. 3. Do not rinse the bottle with sample. Select a clean faucet without attachments or leaking stem. Allow water to run for about ten minutes at full flow from the sampling tap. 4. Reduce flow to avoid splashing, and collect the sample directly into the bottle. Do not use an intermediate container. **Fill to 1" below top of bottle. Cap and invert 5 times to mix sample with preservatives. Carefully add all preservative in vial to sample bottle. Cap the sample and mix sample. Rinse vial and return.**
36CC	1. Do not open the bottle until ready to collect the sample. Do not touch the inside of cap or bottle. 2. Select a kitchen or bathroom sink or a faucet from which water is typically drawn for consumption. **Sampling point should not have been used for a minimum of six (6) hours prior to sampling.** Do not flush the sample tap before sample collection. 3. **Samples must be received in the laboratory within 14 days of collection.**

* **NOTE:** Some tests require thermal preservation. If you received your kit with an ice pack, please ensure that **the ice pack** is frozen prior to return shipment to the laboratory.

EFFECTIVE DATE: 05/2009 SOP# 603 REVISION # 5

ESCHERICHIA COLI IN WATER BY MEMBRANE FILTRATION (NPEC)

Table of Contents

1.0 Applicable Analytical Methods

1.1 *Escherichia Coli (E. coli)* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC), U.S. Environmental Protection Agency, Method 1103.1, 2002.

1.2 *Escherichia Coli (E. coli)* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC), U.S. Environmental Protection Agency, Method 1103.1, 2005.

2.0 Matrix or Matrices

2.1 Recreational waters.

2.2 Surface waters.

2.3 Source-water or Ground water under the direct influence of Surface waters.

3.0 Method Detection Limits

3.1 Not applicable to this method.

4.0 Scope and Application

4.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Escherichia coli* in ambient water. Because the bacteria, is a natural inhabitant only of the intestinal tract of warm-blooded animals, its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens.

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

4.3 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* levels in water can be detected and enumerated.

5.0 Method Summary

5.1 The MF method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter. A water sample is filtered through the membrane which retains the bacteria. After filtration, the membrane containing the bacterial cells is placed on a selective and differential medium, m-TEC, incubated at 35 ± 0.5°C for 2 hours +/-.5 hours to resuscitate injured or stressed bacteria, and then incubated in a water bath at 44.5 ± 0.2°C for 22 ± 2 hours. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 minutes, yellow, yellow-green, or yellow-brown colonies are counted using a magnifying colony counter, if necessary.

6.0 Definitions

6.1 *E. coli* – Those bacteria which produce yellow, yellow-green, or yellow-brown colonies on a filter pad saturated with urea substrate broth after primary culturing on m-TEC medium.

6.2 Colony forming units (CFU) – May consist of single cells or multiple cells in clumps or chains that form single colonies.

7.0 Interferences

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

8.0 Safety

8.1 Eye protection is required in all designated laboratory areas.

8.2 Laboratory coats are required for all bacteriological testing. Additional Personal Protective Equipment, (PPE), requirements can be found in the Hazard Risk Assessment completed on this work area.

8.3 Be familiar with the laboratory chemical hygiene plan.

8.4 Be familiar with the laboratory safety policy (internal SOP 100)

8.5 Exercise caution when operating the autoclave and the Meker burner.

8.6 Mouth-pipetting is prohibited.

8.7 Observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials including disinfecting analytical surfaces and frequent hand washing before performing analysis and when leaving the work area.

9.0 Equipment and Supplies

9.1 Micropipetter - to deliver 10 µL volume

9.2 Magnifying Colony Counter

9.3 Hand tally or electronic counting device

9.4 Microscope 1X-2X dissecting

9.5 Pipettes – Disposable, Sterile, Serological TD 10 mL glass and 1mL plastic 2.5% tolerance

9.6 Membrane filter apparatus for 47 mm filters.

9.7 Vacuum system

9.8 Petri dishes, sterile, plastic, 9 x 50 mm with tight fitting lids.

9.9 47 mm Membrane Filters (approved Gelman GN-6 or Millipore HC).

9.10 Absorbent pads, sterile, 47 mm diameter.

9.11 Incubator capable of maintaining constant temperatures of 35.0 ± 0.5°C.

9.12 Waterbath incubator capable of maintaining constant temperatures of 44.5 ± 0.2°C.

9.13 500 mL autoclavable bottles with caps.

9.14 MIELE Dishwasher-using wash cycle "C". (DEQ Method 917 sec 7.1.1-7.1.5)

9.15 PVC conduit tubes with stoppers – for containment of samples in water bath.

9.16 Glassware – graduated cylinders and volumetric flasks.

9.17 Autoclave – To maintain a temperature of 121°C under 15 psi of pressure for required treatment time.

9.18 Packaging paper, gauze. Integrated Sterilization Indicators, external auto clave thermometers.

10.0 Reagents and Standards

10.1 All reagents, solvents, and standards must be Reagent Grade and traceable to the stock inventory tracking log.

10.2 All reagents, solvents, and standards must be labeled with: date received, date opened, expiration date, tracking number, and receiver's initials.

10.3 All prepared reagents and standards must be labeled with: date prepared, expiration date, preparer's initials, and tracking number.

10.4 All standard logbooks must be completely filled out.

10.5 All certificates of analysis must include the stock inventory tracking number that was assigned to the standard.

10.6 Reagent water – Conforming to specifications in: Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 136 or 141, as applicable), Section 9020 (Reference 21.3).

10.7 Reverse Osmosis (RO) water - Sterile, endotoxin free water is prepared by autoclaving in 500 ml bottles for 35 minutes at 121°C and 15 psi. Sterility checks are performed using commercially purchased 2X Tryptic Soy Broth (TSB). Batches are checked for sterility by combining 50 ml of RO water with 50 ml of 2X TSB and incubating at 35± 0.5°C for 48 hours. If no growth is observed, the batch of RO water is labeled for use with a BW tracking number, and an expiration date of 6 months from date of production. Quality Control paperwork must be filled out and kept as a record for 7 years.

10.8 Tryptic Soy Broth (TSB) - Commercially purchased, sterile single strength at 5 mL volume in tubes and double strength at 50 mL volume in jars. Confirm broth for sterility by placing media in incubator at 35 ± 0.5°C for 48 hours and observing for signs of growth. Broth is confirmed for support of growth by inoculating media with *E.*

coli (ATCC 25922) and observing for growth after 24 hours. pH verified upon receipt should be 7.3 ± 0.2. Product shelf life is as defined by vendor.

10.9 Sodium Hydroxide reagent grade (NaOH), 1 N – Dissolve 40 g of NaOH pellets in reagent water. Dilute to 1 L with reagent water. Label for use with BW tracking number.

10.10 Stock Phosphate Buffer Solution (PBS) – Dissolve 34.0 g potassium dihydrogen phosphate reagent grade in 500 mL of reagent water, adjust to pH 7.2 ± 0.5 with 1 N NaOH, and dilute to 1 L with reagent water. Sterilize by autoclaving at 121°C (15psi) for 30 minutes. Solution is confirmed for sterility by inoculating 50 mL of 2X TSB with 50 mL of solution, incubating at 35.0°C ± 0.5 for 48 hours and observing for growth. Label for use with a BW tracking number. Solution is stored in the refrigerator until used. Discard the solution if signs of mold or turbidity are observed.

10.11 Magnesium Chloride Solution – Dissolve 81.1 g magnesium chloride, reagent grade ($MgCl_2 \cdot 6H_2O$) in 1 L of reagent water. Sterilize by autoclaving at 121°C (15psi) for 30 minutes. Solution is confirmed for sterility by inoculating 50 mL of 2X TSB with 50 mL of solution, incubating at 35.0 ± 0.5°C for 48 hours and observing for growth. Label for use with a BW tracking number. Solution is stored in a refrigerator until used. Discard the solution if signs of mold or turbidity are observed.

10.12 Buffered Rinse Water – Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride solution to 1 L of reagent water. Dispense into containers for use as rinse water. Check for a pH of 7.0+/- 0.2 and record. Sterilize by autoclaving at 121°C, 15 psi for 30 minutes. Batches are checked for sterility by combining 50 mL of buffered rinse water with 50 mL of 2X TSB and incubating at 35.0 ± 0.5°C for 48 hours. If no growth is observed, the batch of rinse water is labeled for use with a BW tracking number Buffered water is stored at room temperature with a shelf life of 6 months.

10.13 Dilution Water Blanks, commercially purchased - Store at room temperature. Dilution water blanks are confirmed for sterility by inoculating 50 mL of 2X TSB with 50 mL Dilution Water Blanks, incubating for 48 hours and observing for growth. pH is recorded upon receipt and should be 7.0 ± 0.2.

10.14 m-TEC agar - Commercially purchased and pre-plated. Plates are stored in a refrigerator until expiration date as defined by the product vendor. pH is verified upon receipt and should be 7.3+/- 0.2. QC requirements for each batch are detailed in Section 12.7

10.15 Urea Substrate medium – Add 10.0 g urea and 0.05 g phenol red to 500 mL reagent water. Stir to dissolve and adjust to pH 3-4 with a few drops of 1N HCl. The substrate solution should be a straw yellow color at this pH and should be stored at 6-8°C for no more than a week.

10.16 Spore Strips - Geobacillus stearothermophilus stored away from light at room temperature. Product shelf life is as defined by vendor.

10.17 Modified TSB - For use with Spore Strips stored away from light at room temperature. Product shelf life is as defined by vendor.

10.18 Control Cultures - The Michigan Department of Community Health (MDCH), Bureau of Laboratory Services, provides the MDEQ, Laboratory Services Section, with some bacteria strains. The laboratory also uses commercially purchased bacterial strains.

10.18.1 Positive Control –
Stock culture of *E-coli* ATCC #25922
E.-coli ATCC #11775 BioBalls (BTF Pty, Sydney, Australia)

10.18.2 Negative Control –
Stock culture of *E. Aerogenes* ATCC #13048
E. Aerogenes ATCC #13048 BioBalls (BTF Pty, Sydney, Australia)
E. faecalis ATCC #19433

11.0 Sample Collection, Preservation, Shipment, and Storage

11.1 Sample containers must be sterile and have a volume of at least 120 mL (Michigan Department of Environmental Quality [MDEQ] Unit 30). They must contain a sufficient amount of sodium thiosulfate to neutralize any disinfectant in the water samples. This is not required if the water is known to be free of disinfectant.

11.2 Ice or refrigerate water samples at < 10°C during transit to the laboratory. Do not freeze samples.

11.3 Sample holding time is 8 hours. Six hours are allowed between collection and initiation of analysis and 2 hours are permitted to set up samples in the laboratory.

12.0 Quality Control (QC)

12.1 Method Blank Control - At the beginning of each analytical batch, filter 50 mL sterile buffered rinse water through a filter and incubate as indicated in Section 14. Data may be rejected if the control is contaminated. The analyst will consult with the Unit Manager to determine if the data are acceptable.

12.2 Prepare the working standard for the negative controls, (*E. aerogenes* ATCC 13048 and E. faecalis 19433) by inoculating one each a tube containing sterile single-strength TSB with culture from the Petri dish containing stock *E. aerogenes and E. faecalis.* The bacteria on the Petri dish may be stressed from refrigeration and the inoculated TSB culture tube should be incubated at 35.0 +/- 0.5 degrees for 2 hours prior to use. Inoculation is performed by transferring a small amount of culture using a sterile loop to the broth and gently swirling it under the liquid media surface.

12.3 Negative Daily Control- (*E. aerogenes* ATCC 13048 and E. faecalis 19433) - At the beginning of each day's analysis, filter a suspension of *E. aerogenes* and E. faecalis and analyze as described in section 14. Suspension will be made by inoculating 2 sterile sample bottles containing 100 mL of sterile R.O. water from negative control working standards, one with 10 uL of viable E-aerogenes and one with10 uL of E. faecalis. Viability of E. aerogenes and E. faecalis will be determined by growth of organism on pre-inoculated non-specific plated media as provided by Michigan Department of Community Health. If the negative control fails to exhibit the appropriate response, reanalyze and/or replace associated media or reagents.

12.4 Prepare the working standard for the positive control, (E. Coli ATCC 29522) by inoculating a tube containing sterile single-strength TSB with culture from the Petri dish containing stock *E-coli.* The bacteria on the Petri dish may be stressed from refrigeration and the inoculated TSB culture tube should be incubated at 35.0 +/- 0.5 degrees for 2 hours prior to use. Inoculation is performed by transferring a small amount of culture using a sterile loop to the broth and gently swirling it under the liquid media surface.

12.5 Positive Daily Control- (*E. coli* ATCC 25922) - At the beginning of each day's analysis, filter a suspension *E. coli* and analyze as is described in section 14. Suspension will be made by inoculating a sterile sample bottle containing 100mL of sterile R.O. with 10 uL of working standard of E-Coli. Viability of organism will be determined by growth of the organism on pre-inoculated non-specific plated media as provided by Michigan Department of Community Health. If the positive control fails to exhibit the appropriate response, reanalyze and/or replace associated media or reagents.

12.6 Initial Demonstration of Capability (IDOC) - Method performance (initial precision and recovery [IPR]) by each laboratory before the method is used for monitoring field samples. EPA recommends but does not require that IDOC analyses be performed by each analyst. IDOC samples should be accompanied by an acceptable method blank (Section 12.1). The IDOC analyses are performed as follows:

12.6.1 Using four commercially purchased dilution blanks inoculate each from a certified concentration of *E.coli* ATCC #11775. Filter and process each IDOC sample according to the procedures in Section 14 and calculate the number of *E. coli* per 100 mL according to Section 15.

12.6.2 Enter colony count data into the laboratory IDOC template. The template will calculate the percent recoveries of the four analyses, the mean percent recovery and the relative standard deviation (RSD) of the recoveries.

12.6.3 Compare the mean recovery and RSD with the corresponding IPR criteria in Table 1, below. If the mean and RSD for recovery of *E. coli* meet acceptance criteria, system performance is acceptable and analysis of field samples may begin. If the mean or the RSD fall outside of the required range for recovery, system performance is unacceptable. In this event, identify the problem by evaluating each step of the analytical process, media, reagents, and controls, correct the problem and repeat IDOC analyses.

Ongoing precision and recovery (OPR) - To demonstrate ongoing control of the analytical system, the lab will periodically analyze dilution blanks spiked with BioBalls containing E. coli ATCC #11775 per section 14. These will be performed as part of the QC necessary for all new lots of plated media and will include the appropriate method blanks, negative controls and sterility checks. Record results in Excel spreadsheet.

Table 1. Initial Precision and Recovery (IPR and OPR) Acceptance Criteria

Performance test	Lab-prepared spike acceptance criteria	BioBall™ spike acceptance criteria

Initial precision and recovery (IPR) • Mean percent recovery • Precision (as maximum relative standard deviation)	76% - 124% 41%	68% - 96% 25%
Ongoing precision and recovery (OPR) as percent recovery	54% - 146%	58% - 106%

12.7 QC sample – QC controls from an outside source are analyzed monthly.

12.7.1 Where more than one analyst is interpreting cultures in a laboratory, the culture count comparison for all analysts must be organized and evaluated by the Unit Manager for each change in personnel or testing procedures. Analysts must be able to duplicate the counts of other analysts within ± 10%. Document results in Excel spreadsheet.

12.7.2 Analysts must be able to duplicate their own counts on the same sample plate within ± 5%. Document results in Excel spreadsheet.

12.7.3 Proficiency testing – A proficiency sample is analyzed on an annual basis.

12.8 Initial Filter Sterility Check- For each new lot of filters, place 1 filter in 50 ml of 2X TSB using sterile forceps and incubate for 48 hours at 35.0 +/- .5 C. Observe for signs of growth. Lot will be designated sterile if there is no growth after 48 hours. Record results on the filter sterility quality control sheet.

12.9 Media QC Check

12.9.1 A QC check must be performed on each new batch of media using a media sterility check and, as required, *E. coli* bacteria , *E. aerogenes and E. faecalis.*

12.9.1.1 The laboratory will test media sterility by incubating one unit (tube or plate) for each new batch of medium (TSB 1X and 2X, m-TEC) as appropriate and observing for growth. Absence of growth indicates media sterility.

12.9.1.2 For each new batch of plated media, a positive control and negative controls will be performed. The positive control will be made by inoculating a sterile dilution blank with BioBall E-Coli ATCC# 11775. Filter the entire dilution blank and analyze as described in Section 14. The negative controls will be made by inoculating 2 bottles containing 100 mL of sterile RO water with 10 uL of working standards of E. aerogenes ATCC#13048 and E. faecalis ATCC# 19433 respectively. Filter 10 mL of each on separate filters and analyze as described in Section 14. Record results. If media fails to exhibit appropriate responses as expected, reanalyze or replace media as necessary.

12.10 Bottle Check - for each lot of sample bottles.

12.10.1 QC checks must be performed by inoculating 3 separate units containing sterile water with one each of MUG-positive *E. coli* strain, a MUG-negative coliform and a non-coliform bacteria, and analyzing each QC check as described in Section 14 of SOP 602.

12.10.2 Bottles are checked for sterility by adding 25 mL of sterile 2X TSB and incubating bottle at 35.0± 0.5C for 48 hours.

12.10.3 The accuracy of the 100 mL mark of each lot of sample bottles must be checked by weighing an empty bottle, adding water to the 100 mL mark, and reweighing the bottle. The weight should equal 100 g ± 2.5 g (1 mL = 1 g) after subtracting the weight of the empty bottle.

13.0 Calibration and Standardization

13.1 Twice daily, check the temperature of the incubators and water bath at a minimum of four hours apart to insure that they are operating within stated limits. If temperatures are outside limits, take corrective action. The temperatures are recorded on the preventative maintenance (PM) sheet provided.

13.2 The temperature of refrigerators shall be checked and recorded daily to insure operation within stated limits. The temperatures are recorded on the PM sheet provided.

13.3 Thermometers shall be recalibrated at least annually with a NIST certified thermometer or replaced annually with a NIST traceable thermometer.

14.0 Procedure

14.1 Place a sterile membrane filter on the filter base, grid side up, and attach the funnel to the base so that the membrane filter is held between the funnel and the base.

14.2 For samples submitted for surface water testing, shake the sample bottle vigorously at least 25 times to distribute the bacteria uniformly. Sample dilutions will be based on the analysis requested by the submitter. Those samples having a test code of NPEC-Lo will be analyzed with two dilutions, (10 ml and 1 ml). Those samples having a test code of NPEC-Hi will be analyzed using 4 dilutions, (10 ml, 1ml, -1, and -2). See Attachment 22.1 for dilution procedures.

NOTE: When analyzing smaller sample volumes (e.g., < 10 mL), 20-30 mL of PBS or phosphate-buffered dilution water should be added to the funnel or an aliquot of sample should be dispensed into a dilution blank prior to filtration. This will allow even distribution of the sample on the membrane.

14.3 Filter the sample, rinsing the sides of the funnel at least twice with 20–30 mL of sterile buffered rinse water. Turn off the vacuum and remove the funnel from the filter base.

14.4 Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the m-TEC Agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane if bubbles occur. Run the forceps around the edge of the filter to be sure that the filter is properly seated on the agar. Close the dish, invert and incubate at 35.0 ± 0.5°C for 2 ± .5 hours in the walk in incubator.

14.5 To reuse the filtration apparatus for another sample, aseptically transfer a sterile filter to the base, hold funnel over a flame for 2-5 seconds, and reassemble funnel in place over filter.

14.6 After the 2 hour incubation, transfer the plates to water tight tubes and place in the water bath incubator at 44.5 ± 0.2°C for 22 ± 2 hours.

14.7 At the end of the incubation time, remove the plates from the water bath. Place an absorbent pad in the lid of the Petri dish and saturate the pad with Urea Substrate Medium. Aseptically transfer the membrane from the m-TEC Agar to the absorbent pad and allow to incubate at room temperature for 15-20 minutes.

14.8 After incubation on the urea substrate, count and record the number of yellow, yellow-green, or yellow-brown colonies on the membrane filters. Select plates/dilutions for each sample that contains a colony count of less than or equal to 100 CFU. If for a given test request, (Hi or Lo), the greatest dilution yields a plate with greater than 100 colonies, than the result reported will be >1,000,000 CFU for the "Hi" test and >10,000 CFU for the "Lo" test. Because the initial dilution for all samples is at 10 mL, those samples containing no method appropriate colonies on the plate after incubation will be reported as "<10 CFU/100 mL."

15.0 Calculations

15.1 Select a membrane filter with an acceptable number of colonies and calculate the number of *E. coli* per 100 mL according to the following general formula.

$$\textit{E. coli} \text{ per 100 mL} = \frac{\text{Number of } \textit{E. coli} \text{ colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

	Volume filtered (mL)	# of colonies	Multiplier	= CFU
	10	20	10	200
	1	20	100	2,000
Dilution: 1 mL to 99 mL:	10 (-1)	20	1000	20,000
Dilution: 1 mL to 99 mL:	1 (-2)	20	10000	200,000

(See Attachment 22.1)

16.0 Method Performance

16.1 All growth and recovery media must be checked to assure that the target organisms respond in an acceptable and predictable manner.

16.2 Specificity - The specificity characteristic of a method is usually reported as the percent of false positive and false negative results. The false positive rate reported for mTEC medium averaged 9% for marine and fresh water samples. Less than 1% of the *E. coli* colonies observed gave a false negative reaction. (Section 21.6)

16.3 Upper Counting Limit (UCL) – That colony count above which there is an unacceptable counting error. The error may be due to overcrowding or antibiosis. The UCL for *E. coli* on m-TEC media has been demonstrated by intra-laboratory testing and analyst comparisons to be 100 colonies per filter.

17.0 Pollution Prevention

17.1 Positive samples must be autoclaved for 15 minutes at 121°C, 15 psi, before disposal. The proper performance of the autoclaves are confirmed weekly using the Spore Strip kit and are monitored with each sterilization batch using sterilization integrated indicator strips and independent autoclave thermometers.

17.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

18.0 Data Assessment

18.1 The data must be qualified for data not analyzed within the sample hold time limits.

18.2 Data may be qualified based on analytical results.

18.3 Samples may be qualified by the analyst.

19.0 Corrective Actions

19.1 Data may be rejected if the negative control sample for a sample series tests positive.

19.2 Data may be rejected if the positive control for a sample series is negative or does not yield results as expected.

19.3 Data may be rejected if the incubator or water bath goes outside the stated limits or if the incubation time is incorrect. The analyst will consult with the Unit Manager to determine if the data is acceptable.

20.0 Waste Management

20.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions. It is also the laboratory's responsibility to protect the air, water and land by minimizing and controlling all releases from bench operations. Compliance with all sewage discharge permits and regulations is also required.

20.2 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is better: Laboratory Chemical Management for Waste Reduction."

21.0 References

21.1 *Escherichia Coli (E. coli)* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC), U.S. Environmental Protection Agency, Method 1103.1, 2002.

21.2 *Escherichia Coli* in Water by the Membrane Filter Procedure, U.S. Environmental Protection Agency, Method 1103.1, 1985.

21.3 APHA, 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. American Public Health Association, Washington D.C.

21.4 Bordner, R., J. A. Winter and P. V. Scarpino (eds.), Microbiological Methods for Monitoring the Environment, Water and Waste, EPA-600/8-78-017. Office of Research and Development, USEPA.

21.5 Test methods for *Escherichia coli* and enterococci in water by the membrane filter procedure, 1985. EPA-600/4-85/076. Environmental Monitoring and Support Laboratory, Cincinnati, USEPA.

21.6 USEPA, 2004. *Results of the interlaboratory Validation of EPA Method 1603 (modified mTEC) for E. coli in Wastewater Effluent.* EPA-821-R-04-020. December 2004.

22.0 Attachment

22.1 Illustration – "Sample Volumes for Filtration and Dilution Preparation."

Signature Page

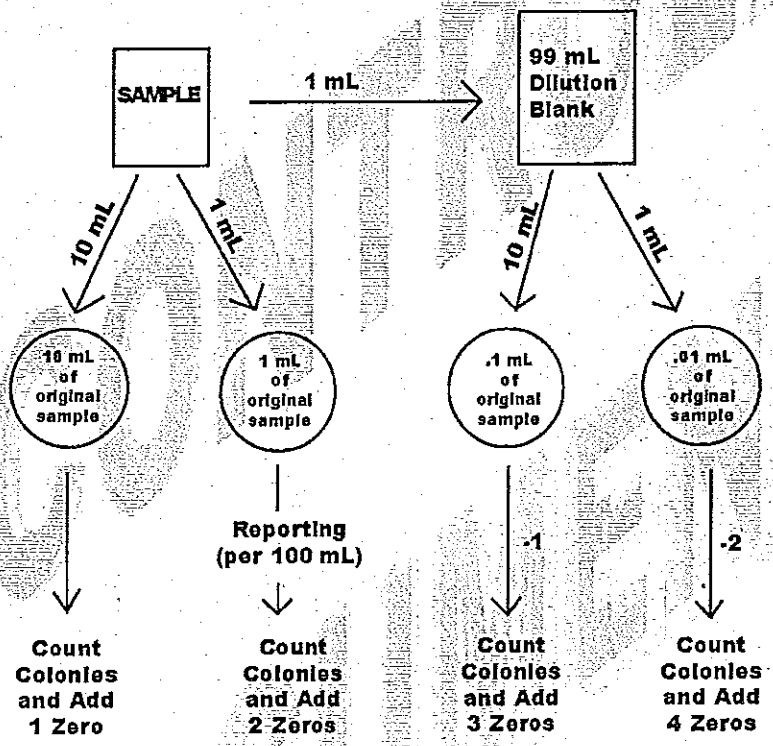
APPROVED BY _____ DATE 5/7/09
CHIEF, LABORATORY SERVICES SECTION

APPROVED BY _____ DATE 5/8/09
COMPLIANCE OFFICER

APPROVED BY _____ DATE 5/7/09
UNIT MANAGER

APPROVED BY _____ DATE 5/7/09
ANALYST

SAMPLE VOLUMES FOR FILTRATION AND DILUTION PREPARATION



RED CEDAR RIVER MONITORING DATA SHEET

Date :_____/_____/_____

Monitor's Name: _____

Current Weather:

❒Clear/Sunny ❒Overcast ❒Showers ❒Rain (Steady) ❒Storm

Worst Weather in Past 48 hrs.:

❒Clear/Sunny ❒Overcast ❒Showers ❒Rain (Steady) ❒Storm

Past 48 Hour Rain Fall Amount: _____ inches

Site ID	GPS Coordinates	Location/Nearest Intersection	Stream Flow (High, Medium, Low)	Temperature (0.0°C)	Time	Channel Depth (cm)	Observations
MSU-							
MSU-							
MSU-							
MSU-							
MSU-							
MSU-							
MSU-							
MSU-							
MSU-							
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