Moore Creek Volunteer Monitoring for *Escherichia coli*

SAMPLING AND ANALYSIS PLAN

Prepared for the Montana Department of Environmental Quality

July 2, 2012

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**Prepared by:**

Katie Kleehammer and Adam Sigler  
MSU Extension Water Quality  
P. O. Box 173120  
Bozeman, MT 59717-3120  

Sunni Heikes-Knapton and William Robertson  
Madison Conservation District  
P. O. Box 606  
Ennis, MT 59729  

**Approvals:**

_________________________________________________________  Date  
Robert Ray (Watershed Protection Section Supervisor)  

_________________________________________________________  Date  
Mindy McCarthy (QA Officer)  

_________________________________________________________  Date  
Adam Sigler (MSU Project Manager)
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Introduction
This document constitutes the Sampling and Analysis Plan (SAP) for the completion of water quality sampling on Moore Creek in the Madison TMDL planning area in Madison County Montana. This effort was initiated by Montana State University Extension Water Quality (MSUEWQ) in collaboration with Sunni Heikes-Knapton, Madison Watershed Coordinator at the Madison Conservation District (MCD) to increase education, outreach, and best management practices amongst landowners in regards to how fecal contamination and *E. coli* can be transported into waterways. Moore Creek originates as a headwater stream in the southern Tobacco Root Mountains before it flows down into the Madison Valley and through Ennis. It has been listed on the 303(d) list for total coliform, and in 2012 it was relisted as impaired for *Escherichia coli* due to the update of standards. MT DEQ will be assessing Moore Creek in July of 2012 to determine if it meets attainment requirements. Attainment requires that the geometric mean of *E. coli* may not exceed 126 colony forming units (cfu)/100mL and no more than 10% of the samples collected within a 30 day period may exceed 252 cfu/100mL between April 1 through October 31 (MT ARM 17.30.623).

This project will not focus on determining if Moore Creek meets attainment. Alternatively, the main goals of this project are threefold: 1) focus on *E. coli* sourcing and determining what land uses may be impacting Moore Creek 2) use the information from the *E. coli* testing to work collaboratively with land owners, MCD, NRCS to assist landowners with improving conditions and riparian areas on their property 3) increase volunteer monitoring capacities and provide additional educational opportunities. This work will be separate from the upcoming Montana DEQ TMDL assessment in the Madison watershed. However, the aim of the project is to assess and potentially address issues before the TMDL process is finished.

The 303(d) list suggests that grazing in riparian or shoreline zones, natural sources and agriculture are potential contributors to the *E. coli* impairment. Through multiple testing sites along the length of Moore Creek from the headwaters to before the confluence with Ennis Lake, it will allow us to better refine our understanding of which areas are continually receiving high concentrations of *E. coli*. This will be accomplished by having volunteer monitors perform several synoptic sampling events along the length of Moore Creek.

Project Objectives
The goals of the project are:

- To develop a watershed *E. coli* educational campaign to increase community awareness about water quality issues in urbanizing areas.
- To heighten awareness among landowners on how *E. coli* and nonpoint source pollution enter our waterways. We will work with interested landowners by pairing with the DNRC and MCD to help assist landowners in assessing their riparian areas and making improvements to keep ungulates out of Moore Creek and to help restore their riparian areas.
To increase volunteer capacity by engaging citizens in water quality data collection to enhance understanding of local water resources and best management practices connected with *E. coli* monitoring.

To enhance the *E. coli* water quality data set for Moore Creek as a tool for educating land owners about water quality issues in urbanizing areas and watersheds with livestock agriculture.

Through the collection of *E. coli* water quality data, the project will provide the following products or opportunities:

- Annual report containing expenses, accomplishments, and any evaluation efforts that have been performed. Annual report will be emailed in PDF format to the DEQ technical project contact.
- Quarterly status reports will be emailed in PDF format to the DEQ technical project contact at the same time that invoices are submitted by the MSU Office of Sponsored Programs.
- At the end of the project, a stand-alone final report in PDF format will be emailed to the DEQ technical contact with a detailed description of efforts for the entire project including limitations, outcomes, impacts, evaluations, data reports, and 319 expenditures. Data generated under the project will be transferred into eWQX according to necessary protocols.
- After data collection the results will be graphed, summarized and potentially plotted in ArcMap. The data will be presented at a public meeting in winter 2012-13 and in the news media in 2013.
- A 5 minute video will be produced including coverage from the monitoring efforts, highlights from the results and information on what people can do to reduce fecal contamination of surface water. The video will be streamed from the MSUEWQ website and will be available to anyone who is interested in a digital copy for presentations.
- Other avenues of results and educational material distribution will also be pursued such as: Big Sky Small Acres magazine, the Montana Ag Live television show and other appropriate newsletters.

**Sampling Design**

The majority of the sample sites along Moore Creek are on private land. The locations of the samples sites were largely based on the cooperation of landowners who granted volunteers permission to access Moore Creek through their property. There are approximately 10 sample sites from the headwaters to the confluence with Ennis Lake (table 2). These sites may be subject to change throughout the season. It is possible that after analyzing the data from several sampling events, we may deem it beneficial to create new sites or retire sites to best suit our goals. Sample sites were selected for the following reasons: changes in land use, changes in property owners and areas open to public access. The majority of the sites are located on private land; only two sites are located on public property. The homeowners of these sites have been contacted and have signed a Property Access Permission Form. Property owners will be notified the day before each sampling event that volunteers will be collecting a sample. The sampling schedule will be focused between June and September of 2012 and 2013.
sampling period, we will be able to capture both the rising and falling limbs on the hydrograph. If possible, we will try to hold at least one sampling event immediately after a precipitation event. Daily precipitation data for 7 days prior to each event will be downloaded from the Western Regional Climate Center’s Remote Automatic Weather station in Ennis, MT to be included with the data analysis and interpretation. The budget analysis is for 100 samples with an additional 20 samples to be used for blanks and duplicates for quality control purposes. The sampling events will be spread out between 2012 and 2013 with the goal of holding five sampling events each year, one a month from May thru September. By spanning the sampling to two years, we also hope to potentially capture any changes in 2013 from any stream side improvements that might be implemented in 2012. The goal of this sampling is not to determine if Moore Creek meets attainment; therefore, we will not be following the sampling schedule guidelines outlined in MT ARM 17.30.623. We will strive to collect 120 samples (including QC samples) during May and September of 2012 and 2013; at a minimum, we will attempt to meet our goal of 80% completeness.

At each site, a volunteer will collect a water sample in a 100 mL sterile bottle from a well-mixed portion of the stream and then transport the sample back to the Madison Conservation District office located in the Lone Elk Mall off of Main St in Ennis, where the sample will be processed for coliform and *E. coli* most probable number (MPN) using the IDEXX Quanti-Tray system. For duplicate samples, the volunteer will be given two samples bottles and will take the samples at the same time and at the same location. Using a co-located approach will emphasize the amount of variability between samples instream. Using a split sample approach would introduce extra steps and bring in additional potential sources of contamination during processing that is not present for the regular samples. A duplicate sample will help to quantify instream variability, where as a split sample approach puts emphasis on quantifying the variability of the enumeration method. A few split samples may be collected to help quantify enumeration variability. Twenty percent of samples for each sampling event will be designated as quality control samples.

MPN is equivalent to cfu/100mL and the two are used interchangeably for recording purposes. “Standards for *E. coli* are based on...most probable number or equivalent membrane filter methods” (ARM 17.30.620). MPN and CFU differ in their methodology, but both methods can detect down to 1 organism per 100mL. MPN is calculated through a series of dilutions which is done automatically with the IDEXX Quanti-Tray system as the Quanti-Tray sealer distributes samples into 97 wells of two different sizes. It is not an absolute number but an estimated mean density of viable colonies in a sample based on probability formulas. Colony forming units (cfu/100mL) is a count of the physical number of colonies that grow on a media plate. The IDEXX system is an EPA approved method for detection of coliforms and *E. coli*.

<table>
<thead>
<tr>
<th>Stream</th>
<th>Parameters</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore Creek</td>
<td>Total coliform, <em>E. coli</em></td>
<td>6 hours</td>
</tr>
</tbody>
</table>

Table 1: 2012 parameters
Figure 1: Sample site locations on Moore Creek
Figure 2: Property ownership along Moore Creek
<table>
<thead>
<tr>
<th>Site ID</th>
<th>Site Name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Site Description</th>
<th>Rational for site selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-HW</td>
<td>Moore Creek Headwaters</td>
<td>45.411588</td>
<td>-111.857506</td>
<td>Site located on USFS property where headwater springs develop on hillslopes</td>
<td>Headwaters site. Most upstream sample site location.</td>
</tr>
<tr>
<td>MC-MP</td>
<td>Moore Creek at Moloney Pond</td>
<td>45.335800</td>
<td>-111.768820</td>
<td>Site located downstream of a pond bed by Moore Creek on the Moloney’s property</td>
<td>Most upstream site on private property that we were granted permission to access.</td>
</tr>
<tr>
<td>MC-MCR</td>
<td>Moore Creek at Moore Creek Road</td>
<td>45.333030</td>
<td>-111.747690</td>
<td>Site is located where Moore Creek crosses Moore Creek Road. The culvert marks the boundary between the Bishop and Collopy properties</td>
<td>Easily accessible site. Site chosen to break up a large stretch of Moore Creek between other sites. The site is also the border line for two different property owners.</td>
</tr>
<tr>
<td>MC-WR</td>
<td>Moore Creek at Willow Ranch</td>
<td>45.336950</td>
<td>-111.741200</td>
<td>Site is located at the entrance to Willow Ranch owned by the Bishop family</td>
<td>Homeowner is interested in potential improvements to the riparian area and would like to monitor to assess conditions and impacts of potential improvements.</td>
</tr>
<tr>
<td>MC-BRK</td>
<td>Moore Creek at Bricker Property</td>
<td>45.338583</td>
<td>-111.737733</td>
<td>Site is located on Dave Bricker’s property</td>
<td>This is a current Madison Stream Team site as well as the last site before Moore Creek travels through the downtown area</td>
</tr>
<tr>
<td>MC-NT</td>
<td>Moore Creek north of town</td>
<td>45.353020</td>
<td>-111.729390</td>
<td>Site is located on the southern end of the Goggin’s Ranch where the stream exits town</td>
<td>Site is downstream of the downtown area. Monitoring here will allow us to assess potential impacts from town.</td>
</tr>
<tr>
<td>MC-FN</td>
<td>Moore Creek at Feed-N-Needs</td>
<td>45.359570</td>
<td>-111.730640</td>
<td>The site is located on the Goggin’s Ranch behind the Feed-N-Needs store, north of downtown Ennis</td>
<td>Site is easily accessible and landowner is interested in current conditions to assess impacts of potential improvements.</td>
</tr>
<tr>
<td>MC-EHS</td>
<td>Moore Creek at Ennis Hot Springs</td>
<td>45.371790</td>
<td>-111.722730</td>
<td>Site is located on Steve Kack’s property just east of Ennis Hot Springs</td>
<td>Site is easily accessible and downstream from actively grazed areas along Moore Creek.</td>
</tr>
<tr>
<td>MC-GOG</td>
<td>Moore Creek near Goggin</td>
<td>45.378700</td>
<td>-111.721883</td>
<td>Site located on Valley Garden Ranch near the property line of the Goggin Ranch</td>
<td>This is an existing Madison Stream Team site that is equipped with a data logger.</td>
</tr>
<tr>
<td>MC-CN</td>
<td>Moore Creek at the confluence</td>
<td>45.406833</td>
<td>-111.709983</td>
<td>Site is located on Valley Garden Ranch property just before the confluence of Moore Creek and the Madison River</td>
<td>Lower most site on Moore Creek before it confluences with the Madison River, also an existing Madison Stream Team site equipped with a data logger.</td>
</tr>
</tbody>
</table>

Table 1: Sample site IDs, names, coordinates and descriptions

*These sites may be subject to change*
Project Team Responsibilities

The project manager will be Adam Sigler, Extension Water Quality Specialist and the project coordinator will be Katie Kleehammer, Water Quality Research Associate with MSUEWQ. Responsibilities of the project manager include project oversight on all components of the project. Responsibilities of the project coordinator include: volunteer recruitment, coordination and trainings, processing samples with IDEXX system, storage/maintenance of equipment, data management, data analysis, report composition. Sunni Heikes-Knapton, Madison watershed coordinator and Robby Robertson, Big Sky Watershed Corps member will be involved with the SAP development, volunteer recruitment, and sample event coordination/assistance. The project administration will be completed by MSUEWQ office, which will include the accounting and financial management of the project. The project team responsibilities are provided in Table 4.

<table>
<thead>
<tr>
<th>Name/Title</th>
<th>Project Responsibilities</th>
<th>Contact information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adam Sigler; MSUEWQ Water</td>
<td>Project Manager: oversight on all components of the project</td>
<td>Sigler Lab, MSU, PO Box 173120, Bozeman, MT, 59717-3120</td>
</tr>
<tr>
<td>Quality Specialist</td>
<td></td>
<td>406.994.7381 <a href="mailto:asigler@montana.edu">asigler@montana.edu</a></td>
</tr>
<tr>
<td>Katie Kleehammer; MSUEWQ</td>
<td>SAP preparation, volunteer recruitment, coordination, and trainings. Sample processing,</td>
<td>Sigler Lab, MSU, PO Box 173120, Bozeman, MT, 59717-3120</td>
</tr>
<tr>
<td>Water Quality Research Associate</td>
<td>data management, and data management training for volunteers.</td>
<td>406.994.7381 <a href="mailto:kkleehammer@montana.edu">kkleehammer@montana.edu</a></td>
</tr>
<tr>
<td>Robby Robertson Big Sky Watershed</td>
<td>Assistance with sample location guide, recruitment, trainings, sample processing</td>
<td>Big Sky Watershed Corps PO Box 606 Ennis, MT 59729</td>
</tr>
<tr>
<td>Corps</td>
<td></td>
<td><a href="mailto:roberwl@tigermail.auburn.edu">roberwl@tigermail.auburn.edu</a> 406-682-3181</td>
</tr>
<tr>
<td>Sunni Heikes-Knapton Madison Watershed</td>
<td>Assistance with SAP development and volunteer recruitment</td>
<td>Madison Watershed Coordinator PO Box 606 Ennis, MT 59729</td>
</tr>
<tr>
<td>Coordinator</td>
<td></td>
<td>406-682-3181 <a href="mailto:mwc@3rivers.net">mwc@3rivers.net</a></td>
</tr>
</tbody>
</table>

Table 3: Project team members and responsibilities

Sampling Methods

Sampling will be conducted according to the standard operating procedures (SOPs) outlined at the end of this document. Site locations have been documented with a Garmin E-Trex GPS. The GPS coordinate system datum is NAD 1983 State Plane Montana, in decimal degrees to the sixth decimal (10cm precision). Photographs of each site: upstream, downstream and cross section views along with driving directions are included in the appendix and will be provided to volunteers for each sample event.
Event and Field methods

Event Coordination and Sample Collection

Volunteers will be recruited through existing Madison Stream Team (MST) volunteer monitor lists, through the high school science teacher, and through word of mouth. The relatively modest training and sampling time requirement will allow us to reach citizens who are interested in volunteer water quality monitoring but not willing to commit the time for the MST summer monitoring efforts. Volunteers will convene at the MCD office before each sampling event and will receive a 30 minute training on why we care about bacteria sampling, what the results do and do not tell us, and how to properly collect a sample. Volunteers will be handed a sheet with photos and driving directions to their sample location, the time the sample is to be collected, sample bottles, gloves, a mini Styrofoam bottle protector, and a sampling stick (if necessary). The sample bottles were purchased from IDEXX and are sterilized and contain sodium thiosulfate which neutralizes free chlorine in the water. See the appendix for sample collection standard operating procedures (SOPs). Once the water has been collected, the volunteer will return to the MCD office and the sample will be immediately processed with the IDEXX quantitray equipment. The sample will then be incubated for 24 hours and trays will be analyzed for most probably number (MPN) of total coliform and \textit{E. coli}. See the appendix for SOPs on IDEXX MPN analytical methods.

Quality control (QC) samples consisting of blanks and duplicates will be collected for 20% of the samples. A field blank is prepared by transporting sterilized (autoclaved) stream water to the field (provided by MSUEWQ) and pouring it into sample containers provided by the lab. The blank will be prepared at the same time that the samples are collected from the stream. A duplicate sample is collected at the same location and time as the original sample. This ensures the sample is collected in the same manner that the regular stream sample is collected. Duplicate and blank samples will be collected at the same site. The site that QC samples are collected at will rotate between events. Duplicate and blank samples are labeled according to the labeling protocol below. Blank and duplicate samples are handled in the same manner that regular samples are handled.

Sample labels should be filled out with Company (MSUEWQ), the date, the time and the sample ID. The sample ID is very important and includes the year, the month, the day, the site ID and a letter indicating they type of sample (regular, blank or duplicate).

Sample ID = Year-Month-Day-SiteID-Sample-Type Letter

A = Regular Sample
B = Duplicate Sample
C = Blank Sample

Sample ID Examples:
A regular sample collected at the Confluence site on June 6\textsuperscript{th}, 2012 would be labeled: 20120611-MC-CNF-A
A duplicate at the same place and time as above: 20120611-MC-CNF-B
Following grab-sample collection, samples will be transported back to the Madison Conservation District office for immediate analysis. The holding time *E. coli* is 6 hours. The samples will be processed well within the holding time.

**Quality Assurance and Quality Control Requirements**

In order for water quality data to be useful, it needs to be an accurate representation of conditions in the water body at the time the samples were collected. This requires proper sample handling and processing and then assessment of data to ensure quality. Data quality objectives (DQOs) state the required quality of data for the intended use and data quality indicators (DQIs) are the specific criteria that data are assessed by to determine quality. Definitions and a list of DQIs are included in the glossary. These indicators are assessed by collecting quality control (QC) samples and then performing quality assurance (QA) checks on those samples.

QC samples are blank and duplicate samples collected in the field for evaluation of quality indicators. Once the results are processed for the QC samples, QA is the process of assessing the data through use of indicators to determine data quality.

**Data Quality Objectives**

Efforts have been made to produce a spatially representative dataset by selecting 10 sites spread over the length of the Moore Creek. However, *E. coli* counts are inherently variable in a stream; this variability will be calculated by looking at the differences between the duplicate samples that are collected at a site. To attempt to reduce the amount of instream spatial variability, volunteers will collect samples from the thalweg, to obtain a well-mixed water sample. See Table 2 for a description of the rational for site selection. Efforts will be made to collect samples from May through September of 2012 and 2013 to capture data from high and low flow events.

Provisions are in place to ensure sensitivity of data collected from the different sample locations and comparability of data collected to other sample events. These provisions include the collection field QC samples and use of the EPA approved IDEXX enumeration method. Data that does not meet quality criteria will be qualified appropriately in the annual report and during the MT EQUIS submission process.

In order to ensure the highest degree of data completeness possible, the project coordinator will check the samples for proper labeling on return to the lab. A minimum of 80% completeness (8 out of 10 scheduled events) is the goal for the project; which accounts for possible weather, access, and volunteer availability challenges.

**Data Quality Indicators**

Quality assurance and quality control (QAQC) can be broken down into a field and a laboratory component. The field component consists of collection of blank and duplicate samples and comparison of data to criteria. The laboratory component consists of assessment of data for blanks as well as a variety of duplicate and spiked samples analyzed by the lab. Blank samples should ideally yield results indicating “no detection”
of the analyte in question. Duplicate samples should ideally produce identical results and analysis of spiked samples should recover exactly the amount of analyte added; however, spiked samples are not conducted for bacteria analysis. Bacteria concentrations are inherently variable, and the criteria outlined in the following two sections are used to assess if data is of acceptable quality.

**Quality Assurance for Field Quality Control Samples**

Field quality control samples will be collected for 20% of all samples collected; this means every 2 in 10 samples will be a QC sample. Because we may not have the same number of volunteers available to collect a grab sample per each sampling event, we will assign 20% of samples per each sampling event to be a QC sample. This will be approximately 2-3 samples per event. Each set of field QC samples will include a blank and a duplicate. Accuracy for field QC samples will be assessed by ensuring that blank samples return a no detection value for coliform and E. coli readings. If a blank sample returns a result greater than a non-detect, the data from that event may need to be qualified. The exception is that data with a value greater than 10 times the detected value in the blank does not need to be qualified. Precision for field QC samples will be assessed by ensuring that relative percent difference (RPD) between duplicates is less than 25%. RPD is calculated using the equation below. In addition to these accuracy/precision checks, it will be necessary to check that all samples were processed within their specified hold times.

**Quality Assurance for Lab Quality Control Samples**

Due to the variability of bacteria sampling, duplicate and spike samples are not traditionally used as quality control measures. However, we will run a lab blank on sterilized stream water. This will check that the sterilized water is in fact sterile and that no outside sources are contaminating the samples which would result in false positive results.

\[
\text{RPD as } \% = \frac{(D1 - D2)}{\left(\frac{(D1 + D2)}{2}\right)} \times 100
\]

Where: D1 is first replicate result, D2 is second replicate result

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Field Blank Threshold</th>
<th>Field Duplicate RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliform</td>
<td>0</td>
<td>&lt; 25% RPD</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td>&lt; 25% RPD</td>
</tr>
</tbody>
</table>

Table 4 Data quality indicator criteria for field QC samples

**Qualifying Data that fails data quality criteria**

If any of the data quality objectives for field or laboratory QC samples fail the criteria above, all data for that analyte for that sample batch must be qualified accordingly. Note that a blank which exceeds the threshold does not automatically mean all data for that sample batch must be qualified. Sample results with values greater than 10 times the detected value in the blank do not need to be qualified. A narrative in the
annual sampling report should outline what data was qualified and for what reason. The data will also need to be qualified during the process of uploading to MT EQUIS using the appropriate qualifier codes. A list of data qualifier codes is provided in the appendix.

**Volunteer Recruitment and Trainings**

A main focus of this volunteer project will be to target pet, livestock and homeowners of septic systems whose actions are directly connected to nonpoint source fecal contamination of water resources. We expect to involve a minimum of 5 volunteers with a hope of recruiting 10 volunteers for each sampling event. The volunteers will be able to help process the water samples using the IDEXX system which is non-intimidating and easy to use. Volunteers will add a Colilert nutrient packet to the sample, transfer the sample to a Quanti Tray, seal the tray with the sealer and incubate the sample. If a volunteer is unable to stay and process the sample, Robby Robertson will handle the sample processing. After 24 hours of incubation, the volunteer can return to assess their Quanti Tray. A yellow colored well indicates coliform presence whereas fluorescence (determined with the help of a black light) indicates *E. coli* presence. The volunteers will count the number of yellow and fluorescing wells to determine the most probable number. See Lab SOPs in the appendix for in-depth directions on this process and picture of a sample Quanti Tray (page 25).

Volunteer recruitment will be done through announcements through the Madison Stream Team list serves, flyers around town and newspaper articles. We will work to engage Ennis High School students for a sampling event during the school session. It will be ideal to have a large enough volunteer base so that there are enough participants to visit each site. If at any of the events we do not have enough volunteers to collect samples from every site, we will still hold the event and sample the most pertinent sites and/or have a few volunteers collect samples at two proximate locations in quick succession. Trainings will be short and succinct, 30-45 minutes and will be held before each sampling event. Returnee volunteers will only need to attend the last 10 minutes to get their site assignment and sample bottles. Trainings will cover general water quality background information with a focus non-point source pollution and *E. coli*. Trainers will familiarize the volunteers with the sample sites with the assistance of GIS maps and photographs of each site. The volunteers will be taught how to properly collect a sample, how to label a sample, how to take a field and lab QC sample, and how to process the samples with the IDEXX equipment. Detailed instructions on sample collection and analysis methods are outlined in the SOP.

**Data Analysis, Record Keeping & Reporting Requirements**

Most probably number (MPN) data for coliform and *E. coli* counts from each sample will be kept in an Excel worksheet that will be maintained by MSUEWQ staff. The MSUEWQ coordinator will review the data to ensure all information is recorded properly (including the QA/QC samples) prior to data entry into MT EQUIS and the VOEIS online database. The results from the IDEXX Quanti-Tray analyses will be double checked by the MSUEWQ coordinator to ensure the MPN is properly recorded. Data qualifiers provided on page 24 will be assigned to data in both hardcopy and electronic form that does not meet these target quality control criteria.
Data generated during this project will be stored on field and lab forms as well as in a master Excel database that MSUEWQ will administer. Written field notes, field forms, and digital photos will be processed by field staff following QA/QC procedures to screen for data entry errors. Data from all sampling events will be entered into EQUIS and VOIES.

The numerical data resulting from this project will not be compared to the water quality standards because we will not be sampling within the required timeframes. However, we will use the numbers in the standards as benchmarks to assess our data as a means of determining what constitutes a high reading (i.e., over 126 cfu). The main purpose of this project is to engage citizens in volunteering and to increase community awareness about water quality issues in urbanizing areas. This project will focus on educating the public and users of Moore Creek about fecal contamination and how \textit{E. coli} and coliforms are transported into our waterways. It will increase volunteer capacity by engaging citizens in water quality data collection in addition to covering best management practices connected with reducing \textit{E. coli} transportation into surface waters.
References


http://www.deq.state.mt.us/wqinfo/QAProgram/WQPBQAP-02.pdf

APPENDIX:

Quality Control Checklist

___ Condition of samples upon receipt
___ Proper collection containers
___ All containers intact
___ All field documentation complete. If incomplete areas cannot be completed, document the issue.
___ Holding times met
___ Field duplicates collected at the proper frequency (specified in SAP)
___ Field blanks collected at the proper frequency (specified in SAP)
___ All sample IDs match those provided in the SAP. Field duplicates are clearly marked on samples and noted as such in lab results.
___ Analyses carried out as described within the SAP (e.g. analytical methods, photo documentation, field protocols)
___ All blanks were less than the project-required detection limit
___ If any blanks exceeded the project-required detection limit, associated data is flagged
___ Laboratory blank samples were analyzed at a rate of 1 per event and returned results below detection
___ Project DQOs and DQIs were met (as described in SAP)
___ Summary of results of QC analysis, issues encountered, and how issues were addressed (corrective action)
___ Completed QC checklist before MT-EQUIS upload
**QA/QC Terms**

**Accuracy.** A data quality indicator, accuracy is the extent of agreement between an observed value (sampling result) and the accepted, or true, value of the parameter being measured. High accuracy can be defined as a combination of high precision and low bias.

**Analyte.** Within a medium, such as water, an analyte is a property or substance to be measured. Examples of analytes would include pH, dissolved oxygen, bacteria, and heavy metals.

**Bias.** Often used as a data quality indicator, bias is the degree of systematic error present in the assessment or analysis process. When bias is present, the sampling result value will differ from the accepted, or true, value of the parameter being assessed.

**Blind sample.** A type of sample used for quality control purposes, a blind sample is a sample submitted to an analyst without their knowledge of its identity or composition. Blind samples are used to test the analyst’s or laboratory’s expertise in performing the sample analysis.

**Comparability.** A data quality indicator, comparability is the degree to which different methods, data sets, and/or decisions agree or are similar.

**Completeness.** A data quality indicator that is generally expressed as a percentage, completeness is the amount of valid data obtained compared to the amount of data planned.

**Data users.** The group(s) that will be applying the data results for some purpose. Data users can include the monitors themselves as well as government agencies, schools, universities, businesses, watershed organizations, and community groups.

**Data quality indicators (DQIs).** DQIs are attributes of samples that allow for assessment of data quality. These include precision, accuracy, bias, sensitivity, comparability, representativeness and completeness.

**Data quality objectives (DQOs).** Data quality objectives are quantitative and qualitative statements describing the degree of the data’s acceptability or utility to the data user(s). They include data quality indicators (DQIs) such as accuracy, precision, representativeness, comparability, and completeness. DQOs specify the quality of the data needed in order to meet the monitoring project's goals. The planning process for ensuring environmental data are of the type, quality, and quantity needed for decision making is called the **DQO process**.

**Detection limit.** Applied to both methods and equipment, detection limits are the lowest concentration of a target analyte that a given method or piece of equipment can reliably ascertain and report as greater than zero.
**Duplicate sample.** Used for quality control purposes, duplicate samples are two samples taken at the same time from, and representative of, the same site that are carried through all assessment and analytical procedures in an identical manner. Duplicate samples are used to measure natural variability as well as the precision of a method, monitor, and/or analyst. More than two duplicate samples are referred to as *replicate samples*.

**Environmental sample.** An environmental sample is a specimen of any material collected from an environmental source, such as water or macroinvertebrates collected from a stream, lake, or estuary.

**Equipment or rinsate blank.** Used for quality control purposes, equipment or rinsate blanks are types of field blanks used to check specifically for carryover contamination from reuse of the same sampling equipment (see field blank).

**Field blank.** Used for quality control purposes, a field blank is a “clean” sample (e.g., distilled water) that is otherwise treated the same as other samples taken from the field. Field blanks are submitted to the analyst along with all other samples and are used to detect any contaminants that may be introduced during sample collection, storage, analysis, and transport.

**Instrument detection limit.** The instrument detection limit is the lowest concentration of a given substance or analyte that can be reliably detected by analytical equipment or instruments (see detection limit).

**Matrix.** A matrix is a specific type of medium, such as surface water or sediment, in which the analyte of interest may be contained.

**Measurement Range.** The measurement range is the extent of reliable readings of an instrument or measuring device, as specified by the manufacturer.

**Method detection limit (MDL).** The MDL is the lowest concentration of a given substance or analyte that can be reliably detected by an analytical procedure (see detection limit).

**Precision.** A data quality indicator, precision measures the level of agreement or variability among a set of repeated measurements, obtained under similar conditions. Relative percent difference (RPD) is an example of a way to calculate precision by looking at the difference between results for two duplicate samples.

**Protocols.** Protocols are detailed, written, standardized procedures for field and/or laboratory operations.

**Quality assurance (QA).** QA is the process of ensuring quality in data collection including: developing a plan, using established procedures, documenting field activities,
implementing planned activities, assessing and improving the data collection process and assessing data quality by evaluating field and lab quality control (QC) samples.

**Quality assurance project plan (QAPP).** A QAPP is a formal written document describing the detailed quality control procedures that will be used to achieve a specific project’s data quality requirements. This is an overarching document that might cover a number of smaller projects a group is working on. A QAPP may have a number of sample analysis plans (SAPs) that operate underneath it.

**Quality control (QC).** QC samples are the blank, duplicate and spike samples that are collected in the field and/or created in the lab for analysis to ensure the integrity of samples and the quality of the data produced by the lab.

**Relative percent difference (RPD).** RPD is an alternative to standard deviation, expressed as a percentage and used to determine precision when only two measurement values are available. Calculated with the following formula:

\[
\text{RPD as } \% = \left( \frac{D1 - D2}{(D1 + D2)/2} \right) \times 100
\]

Where:
- D1 is first replicate result
- D2 is second replicate result

**Replicate samples.** See duplicate samples.

**Representativeness.** A data quality indicator, representativeness is the degree to which data accurately and precisely portray the actual or true environmental condition measured.

**Sample analysis plan (SAP).** A SAP is a document outlining objectives, data collection schedule, methods and data quality assurance measures for a project.

**Sensitivity.** Related to detection limits, sensitivity refers to the capability of a method or instrument to discriminate between measurement responses representing different levels of a variable of interest. The more sensitive a method is, the better able it is to detect lower concentrations of a variable.

**Spiked samples.** Used for quality control purposes, a spiked sample is a sample to which a known concentration of the target analyte has been added. When analyzed, the difference between an environmental sample and the analyte’s concentration in a spiked sample should be equivalent to the amount added to the spiked sample.

**Split sample.** Used for quality control purposes, a split sample is one that has been equally divided into two or more subsamples. Splits are submitted to different analysts or laboratories and are used to measure the precision of the analytical methods.

**Standard reference materials (SRM).** An SRM is a certified material or substance with an established, known and accepted value for the analyte or property of interest.
Employed in the determination of bias, SRMs are used as a gauge to correctly calibrate instruments or assess measurement methods. SRMs are produced by the U. S. National Institute of Standards and Technology (NIST) and characterized for absolute content independent of any analytical method.

**Standard operating procedures (SOPs).** An SOP is a written document detailing the prescribed and established methods used for performing project operations, analyses, or actions.

**True value.** In the determination of accuracy, observed measurement values are often compared to true, or standard, values. A true value is one that has been sufficiently well established to be used for the calibration of instruments, evaluation of assessment methods or the assignment of values to materials.
## Data qualifiers and descriptions

<table>
<thead>
<tr>
<th>Result Qualifier</th>
<th>Result Qualifier Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Detection in field and/or trip blank</td>
</tr>
<tr>
<td>D</td>
<td>Reporting limit (RL) increased due to sample matrix interference (sample dilution)</td>
</tr>
<tr>
<td>H</td>
<td>EPA Holding Time Exceeded</td>
</tr>
<tr>
<td>J</td>
<td>Estimated: The analyte was positively identified and the associated numerical value is the approximate concentration of the analyte in the sample.</td>
</tr>
<tr>
<td>R</td>
<td>Rejected: The sample results are unusable due to the quality of the data generated because certain criteria were not met. The analyte may or may not be present in the sample.</td>
</tr>
<tr>
<td>U</td>
<td>Not Detected: The analyte was analyzed for, but was not detected at a level greater than or equal to the level of the adjusted Contract Required Quantitation Limit (CRQL) for sample and method.</td>
</tr>
<tr>
<td>UJ</td>
<td>Not Detected/Estimated: The analyte was not detected at a level greater than or equal to the adjusted CRQL or the reported adjusted CRQL is approximate and may be inaccurate or imprecise.</td>
</tr>
</tbody>
</table>
Standard Operating Procedures (SOPs)

Field SOPs: Water Sample Collection

- Put on a clean pair of disposable nitrile gloves right before you are about to sample
- Properly label the sample bottle with site name, date, and time with a permanent marker
- Wade into the channel as far as you feel comfortable or sample from the bank and/or use a sample stick if the water is too high to enter.
- Choose a spot that is in the middle of the stream and well-mixed (as long as it is safe to do so), do not sample from eddies or backwater or shallow areas
- Position yourself so that you face upstream
- Break the seal on the bacteria bottle making sure not to touch the inside of the cap or bottle; this will help the bottle to remain sterile and reduce contamination from outside sources.
- Do not rinse the bottle; it contains sodium thiosulphate which neutralizes free chlorine in the water.
- Face the opening of the bottle down towards the stream and dip the sterile bottle about 12 cm below the surface of the water in front of you and fill the bottle to the 100 mL line directly from the stream. – do not rinse the bottle
- Carefully replace the cap and tighten
- Transport the bottles in an insulated package to the Madison Conservation District for analysis. The sample needs to be processed within 6 hours from the collection time.

Lab SOPs: IDEXX E. coli sample analysis

Concept

*E. coli* is a bacteria which is an indicator organism for fecal contamination of water. The amount of *E. coli* present in the water is used to estimate the risk of pathogens being present in the water which can cause disease. IDEXX uses a powdered media which provides food for bacteria and produces color and fluorescence changes when *E. coli* is present in the sample. The Quanti Tray method takes a 100 mL sample and splits it into a number of smaller samples to allow for a calculation of the most probable number (MPN) of bacteria present in the sample.

Equipment List

- Samples
- Colilert media packets
- UV light-6 watt, 365 nm
- Quanti-Tray/2000 (this gives you counts of 1-2,419/100mL without dilution)
- Quanti-Tray/2000 sealer
- Incubator at 35°C ±0.5°C
- Color comparator
- MPN calculation table
- 95% ETOH
• Nitrile gloves

Procedure

1. Turn on the incubator and set it to 35 degrees C.
2. Turn the Quanti-tray sealer on.
3. Clean the counter with 95% ethanol spray.
4. Samples for *E. coli* must be processed the same day they are collected.
5. Samples must be read between 18 and 22 hours (18 hr Colilert) or 24 and 28 hours (24 hr Colilert) from the time they are placed in the incubator.
6. Put on a clean pair of nitrile gloves.
7. Get out enough Quanti Trays for all samples. Use a permanent marker for labeling but not a fine point because it could puncture the trays. Label trays on the back side with a permanent marker with the:
   a. Sample location
   b. Sample date and time
   c. Time the samples are placed in the incubator
8. Lay out one Colilert nutrient packet for each sample next to the bottle.
9. Add the Colilert powder to the bottles and mix for 30 seconds or until the powder is dissolved.
10. Use one hand to hold a Quanti-Tray upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray so that the tray bends toward the palm. If someone assists with holding the tray, they should wear gloves too.
11. Gently pull the foil tab up and out to separate the foil from the tray. Avoid touching the inside of the foil or tray. Avoid separating too far which will cause leakage.
12. Pour the reagent/sample mixture directly into the Quanti-Tray, avoiding contact with the foil tab.
13. Tap the tray to remove air bubbles.
14. Place the sample-filled Quanti-Tray onto the Quanti-Tray rubber insert for the sealer with the well side facing down.
15. Gently push the tray through the Quanti-Tray sealer to seal the tray.
16. Start filling out the *E. coli* datasheet with the sample IDs and the time into the incubator.
17. Place the samples in the incubator and note the time on an IDEXX *E. coli* Lab Data Sheet. Do not stack trays unless necessary.
18. Allow the samples to incubate for 18 to 22 or 24 to 28 hours (depending on the type of Colilert used) before removing them to read.
19. Count the number of small and large cells which have a color equal to or more yellow than the color comparator. Record these numbers on the Lab Data Sheet and use the MPN table to determine the most probably number of total coliform present. Record this number on the Lab Data Sheet along with the times that the samples went into the incubator and were read.
20. Use the UV light box to count the number of small and large cells that fluoresce equal to or more intensely than the comparator. Record these numbers on the Lab Data Sheet and
use the MPN table to determine the most probably number of *E. coli* in the sample. Record this number on the Lab Data Sheet along with the times that the samples went into the incubator and were read.

<table>
<thead>
<tr>
<th>Less yellow than the comparator</th>
<th>Negative for total coliforms and <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow equal to or greater than the comparator</td>
<td>Positive for total coliform</td>
</tr>
<tr>
<td>Yellow and fluorescence equal to or greater than comparator</td>
<td>Positive for coliform and <em>E. coli</em></td>
</tr>
</tbody>
</table>

**Table 1. Result Interpretation Table**

![Image 1: Picture of Quanti Tray](image)

21. After trays have all been checked against datasheets for recording errors, place in autoclave bags and autoclave for 45 minutes at 121°C.
22. The autoclaved bags will then be properly disposed.
Moore Creek Volunteer Monitoring for E. coli Project

Sampling Location Guide

Prepared for the Montana Department of Environmental Quality and Citizen Monitors

May 11, 2012

Prepared By:
William Robertson
Big Sky Watershed Corps Member
Ennis, MT 59729

Katie Kleehammer
MSU Extension Water Quality
P.O. Box 173120
Bozeman, MT 59717-3120
E. coli Sampling Locations along Moore Creek
Madison Valley, MT:
Sample Site 1: Moore Creek at the confluence  
MC – CNF  
(Same as MST site: Moore Lower)

Site Description:

The site is located on Valley Garden Ranch property just before the confluence of Moore Creek and the Madison River. The site is also monitored by the Madison Stream and is outfitted with a data logger. The data logger is circled in Figure 2 and Figure 3. The creek has riparian vegetation along its banks so it may be difficult to spot from afar.

Directions and Parking:

Drive approximately 4 miles north of Ennis to the Valley Garden Ranch entrance at Valley Garden Lane the sampling site is accessed via a ranch road located on the East side of the highway across from the Valley Garden Ranch entrance. This road is lined with planted trees. Turn right at the road, go through the gate, and proceed 0.4 miles east. Turn right at the junction and proceed south onto service road. Be sure not to take the driveway downhill towards the ranch house. The road will go along the crest of a bench and then down the bench to a culvert crossing detention ponds. Continue east 0.3 miles to Moore Creek. Just before crossing the creek, take a left north and follow the rutted trail 0.2 miles to the survey site. Park along the creek at the survey site. Figure 1 can be used for navigation.

Notes:

The site is 50 yards north of four planted trees (circled in Figure 4) and a culvert crossing Moore Creek (look for the data logger rod). The gate code is 3442.
Sample Site 2: Moore Creek near Goggins  
MC – GOG  
( Same as MST site: Moore Middle)

Site Description:
The site is located on the Valley Garden Ranch near the property line of the Goggins Ranch. The site is an existing Madison Stream Team site that is equipped with a data logger rod. This rod can be seen in Figures 3 and 5.

Directions and Parking:
Drive 2.2 miles north out of Ennis and turn right onto a ranch access/service road located just north of the town dump. Go through the red gate and follow the service road down until it ends. Park at the fence line located 50 yards south of power pole (Figure 2). Don’t cross the fence onto the pasture land. Walk 0.25 miles east towards site.

Notes:
Be sure not to confuse MC-GOG with MC-IRS. Both are equipped with data logger rods but the MC-IRS site is only 0.1 miles from parking. MC-GOG is located near the second power pole from parking (Figure 4). Neither site can be seen from the parking lot but both can be identified by the stilling well containing the data loggers.
Sample Site 3: Moore Creek at Ennis Hot Springs  
MC – EHS

Site Description:
The site is located on Steve Kack’s property just east of Ennis Hot Springs.

Directions and Parking:
From Ennis, start heading north on HWY 287. Turn right at KH concrete (across from an abandoned greenhouse) and drive to the end of the road (Figure 1). Enter Ennis Hot Spring through red gate. There will be a shop with two large white doors just beyond the gate (Figure 2). Follow the road until it ends. Park at the wire gate (Figure 3), just above a retention pond for geothermal ground water. Be sure to close the gate behind you. Walk down the road, around the pond to a culvert crossing Moore Creek. Sample downstream of culvert.
Sample Site 4: Moore Creek at Feed N Needs

MC – FN

Site Description:
The site is located on the Goggins Ranch just north of downtown Ennis (Figure 1).

Directions and Parking:
Head north out of Ennis and turn right onto the driveway leading to the Feed N Needs store. The store is located on Goggins Ranch property. Park in the parking lot located outside of the store (Figures 2 and 3). Walk around the store and hay storage (between the highway and the buildings) 50 yards to a red gate (Figure 3) located behind the barn. Open gate and collect sample downstream of bridge crossing Moore Creek (Figure 5). The bridge is located just on the other side of the gate.

Notes:
Be sure to check in at the Feeds N Store to let Mr. Goggins know you have arrived to monitor. Also, cattle may be on the other side of the red gate. Make sure to shut the gate behind you.
Sample Site 5: Moore Creek North of Town

MC – NT

Site Description

The site is located on the southern end of the Goggins Ranch where the stream exits town.

Directions and Parking:

Drive to the Valley Bank in downtown Ennis. Follow the road that runs north in the back parking lot of the bank. Be sure to take the road on the left (bordering the assisted living building) and not the private road on the right. Follow the unpaved road to the brown gate. Continue north to parking area shown in Figure 2. The parking area borders the hospital back packing lot. A large brush pile (circled in Figure 2) is located 50 yards north of parking area. March approximately 100 yards east towards Moore Creek. The site is located at the irrigation structure (Figures 3 and 4) located just north of the neighboring property. Sample above the irrigation structure.
Sample Site 6: Moore Creek at Bricker Property  
MC – BRK  
(Same as MST site: Moore Upper)

Site Description:
The site is located in the backyard of the Bricker family. The site is an established Madison Stream Team site that is marked by a piece of rebar.

Directions and Parking:
Start heading south out of Ennis on highway 287. After passing the post office, turn left on Beaverhead Street and then right on Mirza Way. The address to the Bricker residence is 867 Mirza Way, park in the driveway or along the street (Figure 2). Go around to the backyard and locate the rebar marking the site. The rebar is circled in figures 3 and 4. Take a sample downstream of the bridge.

Notes:
First check in with the Bricker family so they know you are there to monitor.
Sample Site 7: Moore Creek at Willow Ranch

MC – WR

Site Description:

The site is located at the entrance to Willow Ranch. The ranch is owned by the Bishop family. Moore Creek splits on the ranch and converges just before going under the highway to enter town.

Directions and Parking:

Start heading south out of Ennis on highway 287. After just over a mile, turn right onto the entrance to Willow Ranch. There is a place to park to the left of the driveway (Figure 2).

Notes:

Be sure to sample at a point after the two streams have converged but before the water enters the culvert. Sample near the star in figures 3 and 4.
Sample Site 8: Moore Creek at Moore Creek Road

MC – MCR

Site Description

This site is located where Moore Creek Road crosses Moore Creek. The culvert crossing marks the boundary between the Bishop and Collopy properties. The Collopy residence is found atop the driveway next to the parking area. Take a sample downstream of the culvert.

Direction and Parking:

From downtown Ennis, head south on highway 287 towards Virginia City. At 1.5 miles, turn right onto Moore Creek Road. Follow Moore Creek Road until it crosses Moore Creek. Park just beyond the crossing at the fork in the road.
Sample Site 9: Moore Creek at Moloney Pond

MC – MP

Site Description:
The site is located downstream of a pond fed by Moore Creek. The pond is on the Moloney's property.

Directions:
From downtown Ennis, head south on highway 287 towards Virginia City for 1.5 miles. Turn right onto Moore Creek Road. After the road crosses Moore Creek, turn left at the fork onto a dirt road (the fork right after site MC-MCR in figure 1). Follow the dirt road ¾ of a mile and take a left at the next fork. Climb uphill a short distance until you reach the pond. Park along the access road next to the pond (Figure 2). Avoid blocking the driveway to the house. Walk downstream approximately 30 yards and hike off the road to the stream (Figure 3). Look for pink flagging tied to a streamside tree to mark the monitoring site. Collect the sample at the flagged location and do not remove the flagging. Take caution when hiking off the road. There is dense vegetation up to monitoring site that may be challenging to walk through.
Sample Site 10: Moore Creek Headwaters

MC – HW

Site Description:

The site is located at the uppermost spring that contributes to Moore Creek. Sampling should be done just below the spring.

Directions:

The site cannot be accessed by car. Start by heading north out of Ennis. Once you enter McAllister 7 miles later, take a left onto South Meadow Creek road. Follow the road into USFS grounds for 6.4 miles until you reach a junction. Stay to the left and follow the road up the ridge for 3.3 miles to an intersection of two forest service roads. Take a left and continue until you see the trailhead shown in figure 2. Park and begin hiking along the abandoned trail. Hike for approximately 1.25 miles (30 min) until you reach obvious drainage marked by pink flagging. Follow the drainage downhill until you reach spring. Site it marked with a stick stuck in the mud and tied with pink flagging (figure 3).