Clean Water Act Section 319(h) Nonpoint Source Pollution Control Program

Lone Star Healthy Streams
TSSWCB Project Number 06-05
Revision #2

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

prepared by

Texas A&M AgriLife
Texas Water Resources Institute

Effective Period: Upon EPA Approval through September 2010
(with annual updates required)

Questions concerning this quality assurance project plan should be directed to:

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A1 APPROVAL PAGE

Quality Assurance Project Plan for *Lone Star Healthy Streams.*

United States Environmental Protection Agency (EPA), Region VI

Name: Donna Miller
Title: EPA Chief; State/Tribal Programs Section

Signature:_____ Date:_____

Name: Henry Brewer
Title: EPA Texas Nonpoint Source Project Officer

Signature:_____ Date:_____

Texas State Soil and Water Conservation Board (TSSWCB)

Name: Mitch Conine
Title: TSSWCB Project Manager (PM)

Signature:_____ Date:_____

Name: Donna Long
Title: TSSWCB Quality Assurance Officer (QAO)

Signature:_____ Date:_____

Texas A&M AgriLife, Texas Water Resources Institute (TWRI)

Name: Bill Harris
Title: TWRI Acting Director; Project Lead

Signature:_____ Date:_____

Name: Kevin Wagner
Title: TWRI Associate Director, Project Manager (PM)

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Name: Lucas Gregory
Title: TWRI Quality Assurance Officer (QAO)

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Texas AgriLife Extension Service (Extension)—Soil and Crop Sciences (SCSC)

Name: Larry Redmon
Title: Professor and Forage Specialist; Project Co-Lead

Signature:_____ Date:_____

Texas AgriLife Research (Research) – Soil and Crop Sciences (SCSC)

Name: Terry Gentry
Title: Assistant Professor of Soil & Aquatic Microbiology; Lab Director

Signature:_____ Date:_____

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A3 DISTRIBUTION LIST

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

U.S. Environmental Protection Agency Region 6
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Name: Henry Brewer
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Texas State Soil and Water Conservation Board (TSSWCB)
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Name: Donna Long
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Name: Larry Redmon
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Name: Terry Gentry
Tilte: Laboratory Director

List of Acronyms

ACS	American Chemical Society
ARS	USDA-Agricultural Research Service
AWRL	Ambient Water Reporting Limit
BMP	Best Management Practice
CAR	Corrective Action Report
CEU	Continuing Education Units
CFS	Cubic Feet Per Second
CFU	Colony-Forming Unit of Bacteria
COC	Chain of Custody
EMC	Event Mean Concentration
EPA	Environmental Protection Agency
Extension	Texas AgriLife Extension Service
GLCI	Grazing Lands Conservation Initiative
GPS	Global Positioning System
LOQ	Limit of Quantitation
NIST	National Institute of Standards and Technology
NRCS	USDA-Natural Resource Conservation Service
NTU	Nephelometric Turbidity Units
PC	Plum Creek Watershed Site
PCR	Polymerase Chain Reaction
PM	Project Manager
QA	Quality Assurance
QC	Quality Control
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
Research	Texas AgriLife Research
RPD	Relative Percent Difference
rRNA	Ribosomal Ribonucleic Acid
SAML	Soil and Aquatic Microbiology Laboratory
SCSC	Soil and Crop Science Department, Texas A&M University
SM	Standard Methods for Examination of Water and Wastewater, 20^{th} edition
SOP	Standard Operating Procedure
SWCD	Soil and Water Conservation District
TAMU	Texas A&M University
TCEQ	Texas Commission on Environmental Quality
TDA	Texas Department of Agriculture
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas Water Resources Institute
USDA	United States Department of Agriculture
USGS	United States Geological Survey
WWR	Welder Wildlife Refuge Site

A4 PROJECT/TASK ORGANIZATION

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

U.S. Environmental Protection Agency Region 6

Henry Brewer, EPA Texas Nonpoint Source Project Officer

Responsible for managing the project for EPA. Reviews project progress and reviews and approves QAPP and QAPP amendments.

Texas State Soil and Water Conservation Board (TSSWCB)

Mitch Conine, TSSWCB Project Manager

Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Provides the primary point of contact between the TWRI and the TSSWCB. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified in the contract. Notifies the TSSWCB QAO of significant project nonconformances and corrective actions taken as documented in quarterly progress reports from TWRI Project Lead.

Donna Long, TSSWCB Quality Assurance Officer

Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants. Responsible for verifying that the QAPP is followed by the TWRI. Assists the TSSWCB Project Manager on QA-related issues. Coordinates reviews and approvals of QAPPs and amendments or revisions. Conveys QA problems to appropriate TSSWCB management. Monitors implementation of corrective actions. Coordinates and conducts audits

Texas A&M AgriLife, Texas Water Resources Institute (TWRI)

Bill Harris, TWRI Acting Director; Project Lead

The TWRI Project Lead is responsible for ensuring that tasks and other requirements in the contract are executed on time and with the quality assurance/quality control requirements in the system as defined by the contract and in the project QAPP; assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB Project Manager. Responsible for ensuring adequate training and supervision of all activities involved in generating analytical and field data.

Kevin Wagner, TWRI Associate Director, Project Manager

Responsible for coordinating attendance at conference calls, training, meetings, and related project activities with the TSSWCB. Responsible for verifying that the QAPP is distributed and followed by Extension, TWRI, and Research. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions. Responsible for the collection of water samples and field data

measurements in a timely manner that meet the quality objectives specified in Section A7 (Table A7.1), as well as the requirements of Sections B1 through B8. Responsible for field scheduling. Responsible for the acquisition, verification, and transfer of data to the TSSWCB Project Manager. Oversees data management for the project. Performs data quality assurances prior to transfer of data to TSSWCB. Provides the point of contact for the TSSWCB Project Manager to resolve issues related to the data and assumes responsibility for the correction of any data errors. Reports status, problems, and progress to TSSWCB Project Manager.

Lucas Gregory, TWRI Quality Assurance Officer (QAO)

Responsible for coordinating development and implementation of the TWRI's QA program including writing, maintaining and distributing QAPP and any appendices and amendments, and monitoring its implementation. Ensures data collected for the project is of known and acceptable quality and adheres to the specifications of the QAPP. Responsible for identifying, receiving, and maintaining project quality assurance records. Responsible for coordinating with the TSSWCB to resolve QA-related issues. Notifies the TWRI Project Lead, Extension Project Co-Lead, and TSSWCB Project Manager of particular circumstances which may adversely affect the quality of data. Coordinates the research and review of technical QA material and data related to water quality monitoring system design and analytical techniques. Implements or ensures implementation of corrective actions needed to resolve nonconformance noted during assessments. Provides copies of QAPP and any amendments or revisions to each project participant.

Texas AgriLife Extension Service

Larry Redmon, Project Co-Lead

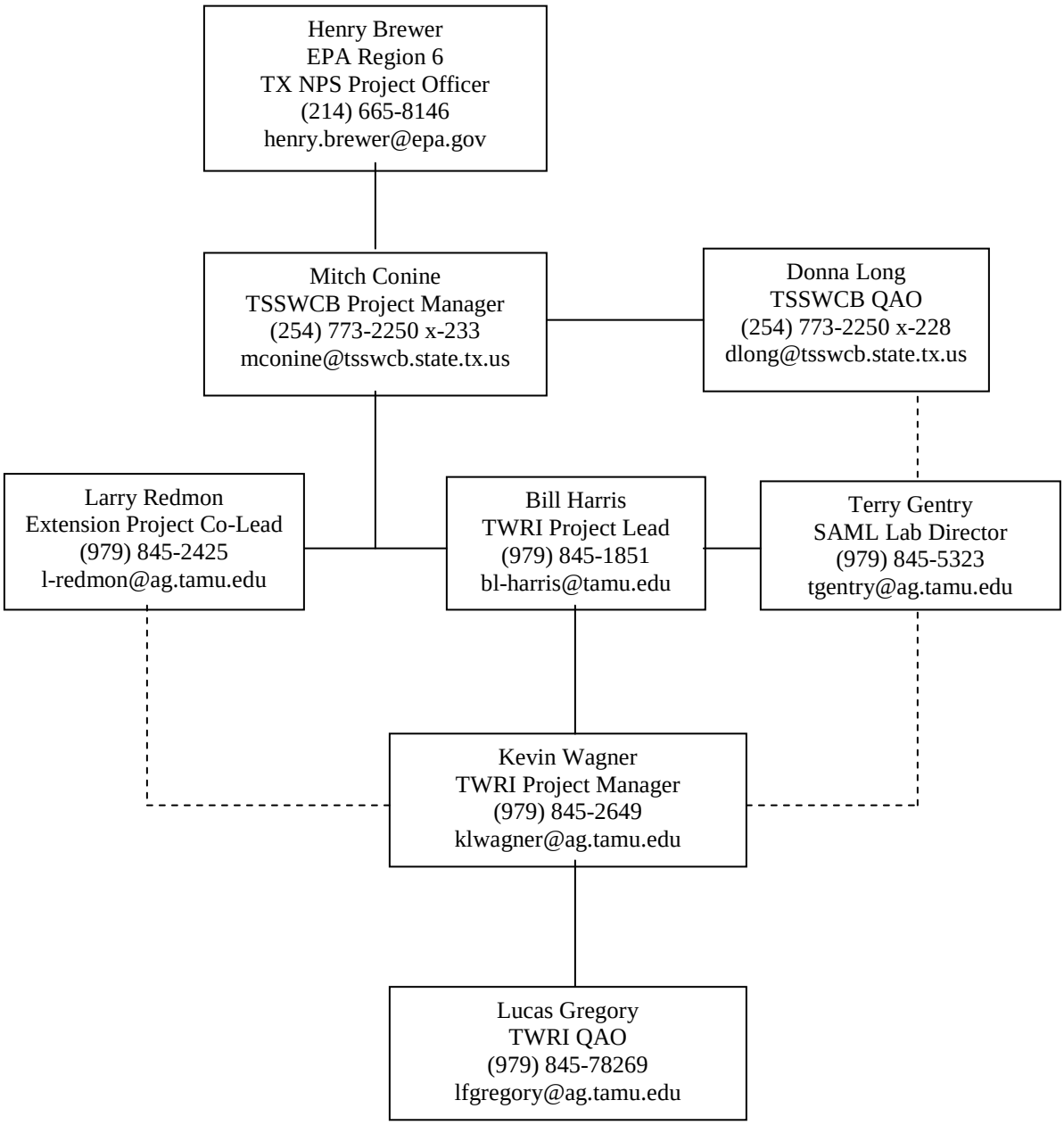
Responsible for verifying that the project is producing data of known and acceptable quality. Responsible for supervising all aspects of the sampling and measurement of surface waters and other parameters in the field. Responsible for field staffing and ensuring that staff is appropriately trained.

Texas AgriLife Research

Terry Gentry, SAML Laboratory Director

Responsible for supervision of laboratory personnel involved in generating analytical data for the project. Responsible for ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and all SOPs specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project data quality objectives in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

Figure A4.1 Organization Chart



A5 PROBLEM DEFINITION/BACKGROUND

According to the *2004 Water Quality Inventory and 303(d) List*, approximately half of the impairments in Texas are the result of excessive bacteria. Bacterial source tracking completed in a number of these waterbodies has identified a noticeable contribution from grazing cattle to the bacterial loading. Grazing lands, which represent the dominant land use in the majority of watersheds in Texas, have received little attention until now regarding the effect of grazing livestock on water quality. Implementation of watershed management principles and practices on grazing lands will be critical to the success of water resource protection efforts in the state in years to come.

Education of landowners and voluntary adoption of BMPs are needed to reduce bacterial contamination of impaired waterbodies. The TSSWCB, local SWCDs and the USDA-NRCS support voluntary adoption of BMPs by producers through technical assistance and cost-share programs.

Extension education programs are designed to target specific audiences and to deliver current, unbiased, science-based information and technology. The objective of the monitoring conducted under this QAPP is to provide the LONE STAR HEALTHY STREAMS Extension education program with unbiased, science-based, quality assured data on the effectiveness of measures for reducing bacteria contamination of streams from grazing lands.

A6 PROJECT/TASK DESCRIPTION

General Project Description

This project is a partnership among the primary federal and state agencies that interface with beef cattle producers relative to environmental management. A Project Steering Committee will be established and coordinated by TWRI to include representatives from the TSSWCB, SWCDs, NRCS, TWRI, Extension, Research, USDA-Agricultural Research Service, TDA, GLCI, Texas Farm Bureau, Texas and Southwestern Cattle Raisers Association, Independent Cattlemen's Association of Texas, Texas Cattle Feeders Association, Welder Wildlife Foundation, Texas Wildlife Association, and independent ranchers. This committee will provide input into evaluation of BMPs, curriculum development, program delivery and CEU processes.

Extension will assess and compile current knowledge regarding BMPs designed to protect grazing lands watersheds from bacteria contamination. Based on this initial task, educational programs and materials will be developed and then tested in priority watershed(s). Concurrent with the development and testing of the educational program, BMPs will be demonstrated and evaluated. BMPs that will be considered for evaluation include, but are not limited to the following: grazing management, shade, fencing, rip-rap, alternative water source development, riparian buffers, and combinations thereof. This evaluation will include an assessment of the effects of these BMPs on cattle behavior, bacterial levels, stream bank stability, and the economic impact of implementing the BMPs on beef cattle producers.

Based on the results of the testing of the education program and BMP demonstration/evaluation, an educational program and associated materials will be developed and delivered state-wide to grazing lands owners and managers in priority watersheds to (1) bring heightened awareness of the issue regarding bacterial contamination of watersheds by grazing animals and (2) to encourage adoption of BMPs designed to reduce bacterial loading to Texas streams and water ways. In order to produce results in a timely manner, the BMP demonstration/evaluation will follow the timeline described in Table A6.1.

Table A6.1. Project Plan Milestones

Task	Project Milestones	Agency	Start	End
1.1	Conduct Annual Project Steering Committee meetings.	TWRI	01/07	09/10
1.2	Prepare & submit quarterly reports to TSSWCB & participants	TWRI	01/07	09/10
1.3	Conduct quarterly meetings with project participants.	TWRI	01/07	09/10
5.1a	Develop QAPP	Extension, TWRI	01/07	08/07
5.1b	Obtain QAPP Approval	TSSWCB, EPA	08/07	09/07
5.2	QAPP Annual Revision #1	Extension, TWRI	08/08	11/08
5.2	QAPP Annual Revision #2	Extension, TWRI	11/09	11/09
5.3a	Identify cooperator for alternative water BMP demonstration	Extension, TWRI	01/07	05/07
5.3b	Identify cooperator for grazing management demonstration	Extension, TWRI	01/07	05/07
5.4	Assess pre & post BMP cattle behavior using GPS collars	Extension, TWRI	08/07	08/09
5.5a	Install sampling equipment at demonstration sites	Extension, TWRI	05/07	10/07
5.5b	Conduct stormwater bacteria sampling for grazing demo	Extension, TWRI	09/07	08/10
5.5c	Conduct bi-monthly bacteria sampling and flow evaluation for alternative water BMP demonstration	Extension, TWRI	08/07	08/09
5.6	Conduct stormwater *Bacteroides* sampling for grazing demo	Extension, TWRI	09/07	08/10
5.7	Install alternative water BMP	Extension, TWRI	09/08	10/08
5	Compile and analyze sampling data	Extension, TWRI	08/10	09/10
5	Develop report describing demonstration results	Extension, TWRI	08/10	09/10
5	Develop fact sheet describing demonstration results	Extension, TWRI	08/10	09/10

Evaluation of Best Management Practices

Effects of grazing management will be evaluated over a period of 2 years using runoff samples from three 1-ha watershed sites located at the Welder Wildlife Refuge (WWR-1, 2, 3) and two 1.2-ha sites located at the USDA-ARS Grassland Soil and Water Research Laboratory near Riesel (SW-12, SW-17). On the Welder Wildlife Refuge, WWR-1 will be ungrazed rangeland, WWR-2 will be moderately grazed rangeland, and WWR-3 will be heavily grazed rangeland. At Riesel, SW-12 is an ungrazed native prairie reference site and SW-17 is a moderately grazed coastal bermudagrass pasture. Rainfall depth, rainfall intensity, and flow will be measured for each event. Turbidity and event mean concentrations for *E. coli* and *Bacteroides* will be determined for each runoff event where sufficient sample volume is available. *Enterococcus* will also be evaluated on selected events.

Alternative water supplies will be evaluated over a period of 2 years utilizing bi-monthly inflow and outflow samples from a cooperating ranch located on Clear Fork of Plum Creek. During year 1, no alternative water will be provided to the cattle. In year 2, alternative water will be provided. All water samples will be analyzed for *E. coli* and turbidity. Flow will be determined for each sample event and days since last rain will be documented. Each quarter, cattle movement will be tracked using GPS collars allowing assessment of the effect of providing alternative water. The percent time cattle spend within the riparian zone of the stream will be assessed using this technology both before and after the practice is installed. Quarterly assessment will allow evaluation of seasonality. Stream stability will be evaluated at least semi-annually by the establishment of permanent cross sections and photodocumentation.

E. coli will be analyzed by the Soil and Aquatic Microbiology Laboratory (SAML) using EPA Method 1603 [EPA (2005). Method 1603: *Escherichia coli* (*E. coli*) in water by membrane

filtration using modified membrane-thermotolerant *Escherichia coli* agar (Modified mTEC). Washington, DC, Office of Research and Development, Government Printing Office]. *Enterococcus* will be analyzed by the SAML using EPA Method 1600 [EPA (2002). Method 1600: *Enterococci* in Water by Membrane Filtration Using membrane-*Enterococcus* Indoxyl-ß-D-Glucoside Agar (mEI). EPA-821-R-02-022. Washington, DC, Office of Water, Government Printing Office]. Polymerase chain reaction (PCR) genetic testing for *Bacteroides* fecal bacteria will be performed by SAML to determine the source of the fecal pollution. The *Bacteroides* PCR method is a culture-independent molecular method which targets genetic markers of *Bacteroides* and *Prevotella* spp. fecal bacteria that are specific to humans, ruminants (including cattle and deer), pigs, and horses **[**Bernhard, A. E. and K. G. Field (2000). "A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA." Appl Environ Microbiol 66(10): 4571-4574**;** Dick, L. K., A. E. Bernhard, et al. (2005). "Host distributions of uncultivated fecal Bacteroidales bacteria reveal genetic markers for fecal source identification." Appl Environ Microbiol 71(6): 3184-3191**].** The method has high specificity and moderate sensitivity **[**Field, K. G., E. C. Chern, et al. (2003). "A comparative study of culture-independent, library-independent genotypic methods of fecal source tracking." J Water Health 1(4): 181-94**]**. For this method, 100 ml water samples are concentrated by filtration, DNA extracted from the concentrate and purified, and aliquots of the purified DNA analyzed by PCR. Results are expressed as number of host-specific organisms per 100 ml. Percent contribution of each host-specific *Bacteroides* to the total *Bacteroides* detected for each sample will be estimated. For pre-processing of water samples for *Bacteroides* PCR, water samples will be filtered and the filters placed in DNA lysis buffer and frozen at -80°C until analyzed. At the time of analysis, the Soil and Aquatic Microbiology Lab will extract and purify DNA from the filters. Extracted DNA will be tested for ruminant (including cattle and deer), pig (including feral hogs), and other fecal markers as described by Layton, A. L. McKay, et al. (2006). "Development of Bacteroides 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water." Appl Environ Microbiol 72(6): 4214-4224.

A7 QUALITY OBJECTIVES AND CRITERIA

The project objective is to evaluate and demonstrate the effectiveness of BMPs in reducing bacterial contamination from grazing lands. BMPs that will be evaluated include grazing management and alternative water source development. The measurement performance specifications to support the project objective are specified in Table A7.1.

Ambient Water Reporting Limits (AWRLs)

The AWRL establishes the reporting specification at **or below** which data for a parameter must be reported to be compared with freshwater screening criteria. The AWRLs specified in Table A7.1 are TCEQ CRP program-defined reporting specifications for each analyte. The limit of quantitation (formerly known as the reporting limit) is the minimum level concentration, or quantity of a target variable (e.g., target analyte) that can be reported with a specific degree of confidence.

- The laboratory's Limit of Quantitation (LOQ) for each analyte must be at **or below** the AWRL as a matter of routine practice

- The laboratory must demonstrate its ability to quantitate at its LOQ for each analyte by running an LOQ check standard each time that samples are analyzed.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria are provided in Section B5.

Precision

The precision of laboratory data is a measure of the reproducibility of a result from repeated analyses. It is strictly defined as a measure of the closeness with which multiple analyses of a given sample agree with each other. Precision is assessed by repeated analyses of a sample. For quantitative microbiological analyses, the method to be used for calculating precision is the one outlined in Standard Methods for the Examination of Water and Wastewater, 20th Edition, section 9020 B.8.b.

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

The $RPD_{bacteria}$ should be lower than $3.27 * \Sigma Rlog/n$, where Rlog is the difference in the natural log of duplicates for the first 15 positive samples.

Laboratory precision is assessed by comparing sample/duplicate pairs, in the case of bacterial analysis. Precision results are compared against measurement performance specifications and used during evaluation of analytical performance. Measurement performance specifications for precision are defined in Table A7.1.

Bias

Bias is a statistical measurement of correctness and includes components of systemic error. A measurement is unbiased when the value reported does not differ from the true value. Bias is determined through the analysis of laboratory control standards and LOQ check Standards prepared with verified and known amounts of all target analytes in the sample matrix (e.g. deionized water, commercially available tissue) and by calculating percent recovery. Results are compared against measurement performance specifications and used during evaluation of analytical performance. Performance specifications for bias are specified in Table A7.1.

An additional element of bias is the absence of contamination. This is determined through the analysis of field blank samples of sterile water taken to the field and processed in a manner identical to the sample. Requirements for field blank samples are discussed in Section B5.

Representativeness

Data collected under this project will be considered representative of ambient water quality conditions. Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions typical of a receiving water. The representativeness of the data is dependent on 1) the sampling locations, 2) the number of samples collected, 3) the number of years and seasons when sampling is performed, 4) the number of depths sampled, and 5) the sampling procedures. Site selection procedures will assure that the measurement data represent the conditions at the site. The goal for meeting total representation of the water body and watershed is tempered by the availability of time, site accessibility, and funding. Representativeness will be measured with the completion of sample collection in accordance with the approved QAPP.

Table A7.1. Measurement Performance Specifications

PARAMETER	UNITS	METHOD	LOQ	Precision of Laboratory Duplicates	Bias	Percent Complete
Field Parameters						
Days since last rain	*days*	*TCEQ SWQM Procedures*	*NA*	*NA*	*NA*	*90*
Flow Depth	*inches*	*TCEQ SWQM Procedures*	*NA*	*NA*	*NA*	*90*
Estimated Flow	*cfs*	*Manning's Equation*	*NA*	*NA*	*NA*	*90*
Lab Parameters						
Turbidity	*NTU*	*SM 2130-B*	*0.5*	*80-120*	*NA*	*90*
E. coli	*cfu/100 ml*	*EPA 1603*	*1.0*	*3.27 * ΣRlog/n*	*NA*	*90*
Enterococci	*Cfu/100 ml*	*EPA 1600*	*1.0*	*3.27 * ΣRlog/n*	*NA*	*90*
Bacteroides PCR	*orgs/100ml*	*Extraction = EP AREC SOP PCR = Layton et al. 2006*	*NA*	*100% agreement*	*90% correct*	*90*

Comparability

The comparability of the data produced is predetermined by the commitment of the staff to use only approved procedures as described in this QAPP. Comparability is also guaranteed by reporting all ambient, high flow, and QC data for evaluation by others.

Completeness

The completeness of the data is a measure of how much of the data is available for use compared with the total potential data. Ideally, 100% of the data would be available. However, the possibility of unavailable data due to accidents, weather, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 90 percent data completion is achieved.

A8 SPECIAL TRAINING/CERTIFICATION

No special certifications are required. However, field personnel will receive training in proper sampling. Before actual sampling occurs, field personnel will demonstrate to the Extension Co-Lead, TWRI PM, and TWRI QAO their ability to properly perform field sampling procedures. Laboratory analysts have a combination of experience, education, and training to demonstrate knowledge of their function. To perform analyses for the TSSWCB, each laboratory analyst must demonstrate their capability to conduct each test that the analyst performs to the Lab Director. This demonstration of capability is performed before analyzing samples and annually thereafter.

A9 DOCUMENTS AND RECORDS

The document and records that describe, specify, report, or certify activities, requirements, procedures, or results for this project and the items and materials that furnish objective evidence of the quality of items or activities are listed in Table A9.1.

Table A9.1 Project Documents and Records

Document/Record	Location	Retention	Form
QAPP, amendments, and appendices	TWRI	5 years	Paper/Electronic
Field notebooks	Extension	5 years	Paper
Chain of custody records	TWRI	5 years	Paper
Corrective action reports	TWRI	5 years	Paper
Bacteriological data log sheet	SAML	5 years	Paper
Laboratory QA manuals	SAML	5 years	Paper
Laboratory SOPs	SAML	5 years	Paper
Instrument raw data files, readings and printouts	SAML	5 years	Paper/Electronic
Lab equipment calibration records & maintenance logs	SAML	5 years	Paper
Lab data reports	TWRI/TSSWCB	3 years	Paper/Electronic
Progress reports/final report/data	TWRI/TSSWCB	3 years	Paper/Electronic

Quarterly progress reports will note activities conducted in connection with the water quality monitoring program, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP. All quarterly progress reports and QAPP revisions will be distributed to personnel listed in Section A3.

All electronic data are backed up on an external hard drive monthly, compact disks weekly, and is simultaneously saved in an external network folder and the computer's hard drive. A blank CAR form is presented in Appendix A, a blank COC form is presented in Appendix B, and blank bacteriological data log sheet is presented in Appendix C.

The TSSWCB may elect to take possession of records at the conclusion of the specified retention period.

QAPP Revision and Amendments

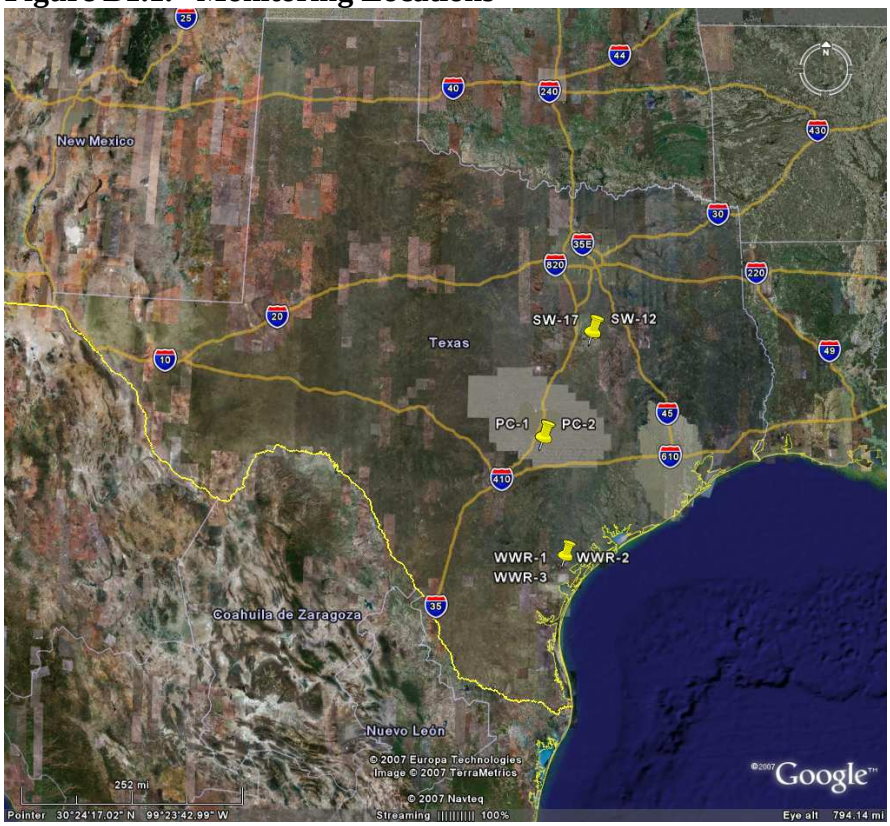
Until the work described is completed, this QAPP shall be revised as necessary and reissued annually on the anniversary date, or revised and reissued within 120 days of significant changes, whichever is sooner. The last approved versions of QAPPs shall remain in effect until revised versions have been fully approved; the revision must be submitted to the TSSWCB for approval before the last approved version has expired. If the entire QAPP is current, valid, and accurately reflects the project goals and the organization's policy, the annual re-issuance may be done by a certification that the plan is current. This will be accomplished by submitting a cover letter stating the status of the QAPP and a copy of new, signed approval pages for the QAPP.

QAPP amendments may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and nonconformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances. Written requests for amendments are directed from the TWRI PM or TWRI QAO to the TSSWCB PM and are effective immediately upon approval by the TSSWCB PM and QAO, and EPA Project Officer. Amendments to the QAPP and the reasons for the changes will be documented and distributed to all individuals on the QAPP distribution list by the TWRI QAO. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

B1 SAMPLING PROCESS DESIGN

The goal of the sampling is to evaluate BMPs to determine their effectiveness for reducing bacteria and then provide landowners with this science-based assessment. To achieve this goal, data collection efforts will involve monitoring both edge of field and in-stream water quality data for the purpose of aiding evaluation of BMP effectiveness in reducing bacteria loadings under various scenarios. Best management practices will be evaluated at three locations: the Welder Wildlife Refuge located in the Copano Bay watershed, the USDA-ARS Grassland Soil and Water Research Laboratory near Riesel, and a private ranch located on the Clear Fork of Plum Creek near Lockhart.

Figure B1.1. Monitoring Locations



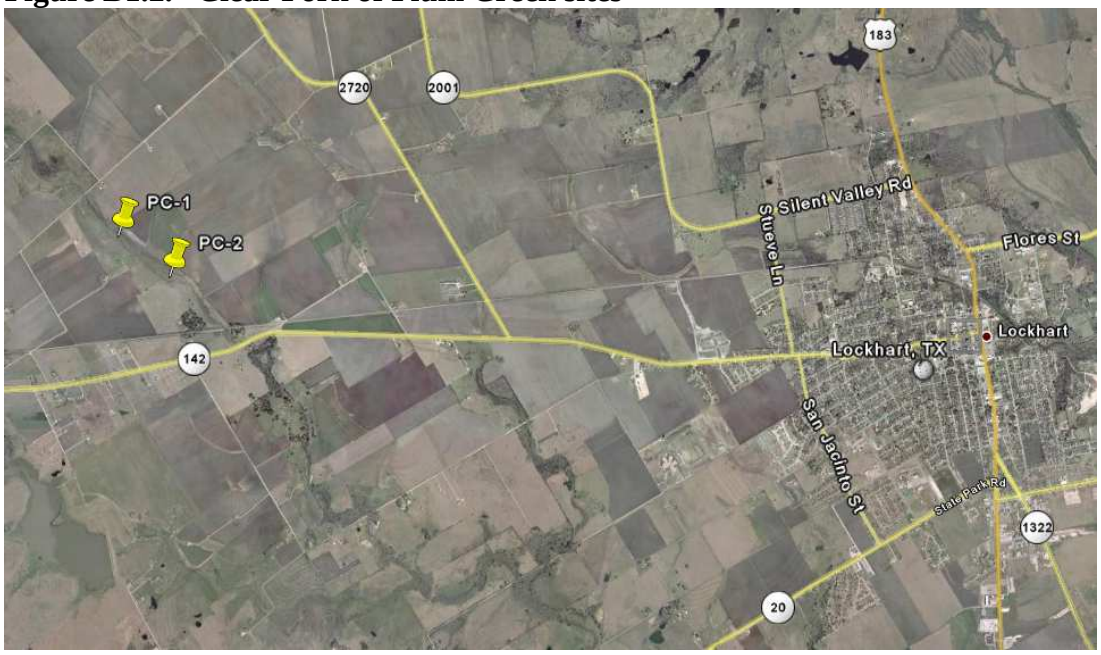
Monitoring will encompass evaluating water quality parameters that indicate bacterial contamination, isolating bacteria collected, and determining the source of said bacteria. Information gained in this portion of the project will be used to educate landowners concerning bacterial impairments and effectiveness of BMPs focused on reducing potential contamination sources. The constituents that will be measured are shown in Table B1.1.

Table B1.1. Waterborne Constituents

Parameter	Status	Reporting Units
Escherichia coli	Critical	cfu per 100 milliliters (cfu/100 ml)
Enterococci	Noncritical	cfu per 100 milliliters (cfu/100 ml)
Bacteroides	Critical	Organisms per 100 milliliters (orgs/100 ml)
Turbidity	Critical	Nephelometric Turbidity Units (NTU)
Flow	Critical	cubic feet per second (cfs)
Water depth	Critical	inches (in)

Two sites (inflow and outflow) on the cooperating ranch on Clear Fork of Plum Creek will be monitored to assess the effectiveness of alternative water sources (Figure B1.2). During the first year, no alternative water will be provided. During the second year, alternative water will be provided. Routine grab samples will be collected and analyzed on a biweekly basis when water is flowing at sampling sites. Any sites found to be dry or with pooled water will be noted in the field notebook. Flow depth will also be measured in order to determine flow.

Figure B1.2. Clear Fork of Plum Creek sites



In order to obtain representative results, ambient water sampling will occur on a routine schedule over the course of 24 months, capturing dry and runoff-influenced events at their natural frequency. There will be no prejudice against rainfall or high flow events, except that the safety of the sampling crew will not be compromised in case of lightning or flooding. Permanent cross sections will be established and measured semi-annually to assess impacts of BMP implementation on streambank stability. In addition, cattle behavior will be assessed quarterly to evaluate the impacts of BMP implementation on the percent time that cattle spend within the stream and its riparian zone.

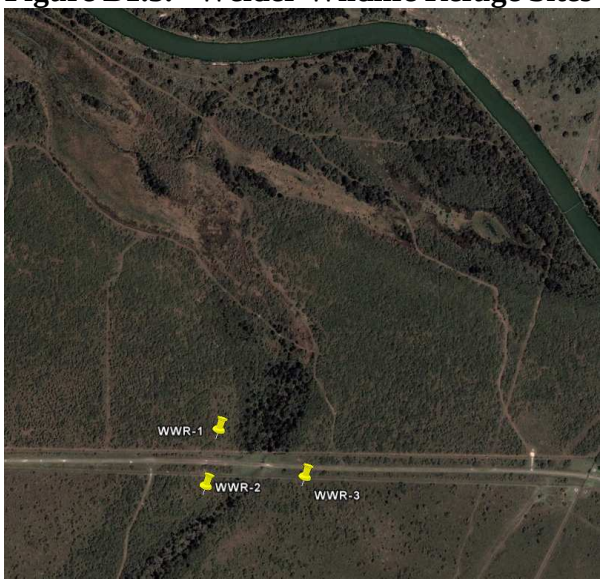
In the instance that a sampling site (Table B1.2.) is inaccessible, no sample will be taken and will be noted in the field notebook. If, near the end of the study, the TSSWCB PM and QAO agree that the sampling has not achieved good representativeness of typical conditions, they may restrict the final sampling event(s) to target a particular environmental condition (e.g., rainfall).

Table B1.2. Sample Sites and Monitoring Frequencies

Station ID	Long Description (lat/long)	Monitoring Frequencies (per fiscal year) for each Parameter Group				
		Turbidity	*E. coli*	*Bacteroides*	*Enterococci*	Flow
WWR-1	28° 6'55.97"N / 97°21'20.82"W	Runoff events	Runoff events	Runoff events	Selected runoff	Runoff events
WWR-2	28° 6'51.98"N / 97°21'21.89"W	Runoff events	Runoff events	Runoff events	Selected runoff	Runoff events
WWR-3	28° 6'52.60"N / 97°21'13.83"W	Runoff events	Runoff events	Runoff events	Selected runoff	Runoff events
SW-12	31° 28’48”N / 96° 52’59”W	Runoff events	Runoff events	Runoff events	Selected runoff	Runoff events
SW-17	31° 27’45”N / 96° 53’14”W	Runoff events	Runoff events	Runoff events	Selected runoff	Runoff events
PC-1	29°53'35.81"N / 97°45'21.06"W	Bi-monthly	Bi-monthly	NA	NA	Bi-monthly
PC-2	29°53'23.28"N / 97°45'2.67"W	Bi-monthly	Bi-monthly	NA	NA	Bi-monthly

Three sites (WWR 1, 2, 3) will be monitored on the Welder Wildlife Refuge (Figure B1.3) to evaluate effects of grazing management on bacteria runoff. Each watershed site is 1 hectare in size and equipped with berms and v-notch weirs to aid in collection and measurement of runoff. At each site, an ISCO bubble flow meter and sampler is installed to measure flow and collect runoff. An ISCO rain gage will measure rainfall depth and intensity. ISCO samplers will be programmed to collect flow-weighted composite samples allowing determination of event mean concentrations (EMCs) for *E. coli* and *Bacteroides* for each rain event. *Enterococci* will also be evaluated for selected events. Site WWR-1 will be ungrazed throughout the study. Site WWR-2 will receive moderate grazing intensity (1 animal unit / 14 acres). Site WWR-3 will receive heavy grazing intensity (1 animal unit / 7 acres).

Figure B1.3. Welder Wildlife Refuge Sites



Two sites (SW-12, SW-17) will be monitored on the USDA-ARS Grassland Soil and Water Research Laboratory near Riesel (Figure B1.4) to evaluate effects of grazing management on bacteria runoff. Each watershed site is 1.2 hectares in size and equipped with berms and weirs to aid in collection and measurement of runoff. At each site, an ISCO bubble flow meter and sampler is installed to measure flow and collect runoff. An ISCO rain gage will measure rainfall depth and intensity. ISCO samplers will be programmed to collect flow-weighted composite samples allowing determination of event mean concentrations (EMCs) for *E. coli* and *Bacteroides* for each rain event. *Enterococci* will also be evaluated for selected events. SW-12 is an ungrazed native prairie reference site and SW-17 is a moderately grazed coastal bermudagrass pasture.

Figure B1.4. USDA-ARS Research Lab at Riesel Sites

B2 SAMPLING METHODS

Edge of Field Sampling Procedures

Flow-weighted composite edge of field samples from the three watershed sites located at the Welder Wildlife Refuge will be collected using ISCO 6712 full-size portable samplers with single bottle configuration into sterile polyethylene 4-gallon round bottles (Table B2.1). This will allow calculation of event mean concentrations of bacteria for each rainfall event. Flow from each watershed site will be measured with ISCO 730 Module bubble flow meters. This, in combination with the EMCs, will allow calculation of bacteria loading for each rainfall event. Rainfall depth and intensity will be measured by and ISCO 674 rain gage. Rainfall and flow data will be downloaded weekly using an ISCO 581 Rapid Transfer Device (RTD). This will allow association of loadings with varying magnitudes of rainfall events.

In-Stream Sampling Procedures

In-stream sampling will be conducted on a bi-monthly basis for two years at the two sites on Clear Fork of Plum Creek to evaluate providing alternative water. Water samples will be collected directly from the stream (midway in the water column) into sterile Whirlpack® bags (Table B2.1). The sample container will be held upstream of the sampler and care will be exercised to avoid contact with sediment and the surface micro layer of water, which may be enriched with bacteria and not representative of the water column. The top one inch of water will be squeezed from the bag before whirling and sealing. This airspace will help mix the sample when it is shaken just before making dilutions and membrane filtration.

Table B2.1. Field Sampling and Handling Procedures

Parameter	Matrix	Container	Preservation	Sample Volume	Holding Time
Turbidity	Water	Sterile bacteriological bottles / Whirlpack® bags	4°C	5 ml	48 hours
E. coli	Water	Sterile bacteriological bottles / Whirlpack® bags	4°C	25 ml	8 hours/48 hours[1]
Enterococci	Water	Sterile bacteriological bottles / Whirlpack® bags	4°C	25 ml	48 hours[1]
Bacteroides	Water	Sterile bacteriological bottles	4°C	100 ml	48 hours[1]
MIN. NEEDED	**Water**	**Sterile bacteriological bottles / Whirlpack® bags**	**4°C**	**155 ml**	**8 hours/48 hours**

[1] An 8-hour holding time will be met for all in-stream *E. coli* samples consistent with EPA 1603. A 48 hour holding time will be met for all runoff *E. coli*, *Enterococci*, and *Bacteroides* samples as described below.

Holding Time

In a study funded by EPA, Pope et al. concluded that *E. coli* samples analyzed beyond 8 hours after sample collection still generate comparable *E. coli* data, provided that the samples are held below 10^{o}C and are not allowed to freeze. Pope reported a majority of sites showed no significant differences in *E. coli* densities between the 0-hour and the 48-hour holding times. Additionally, Pope reported, a majority of *E. coli* samples held at 20 and 35^{o}C showed no significant difference at the 8-hour holding time compared to the 0-hour results. [Applied and Environmental Microbiology, Oct. 2003, pp. 6201-6207]

Stormwater samples from edge-of-field watershed sites at Riesel and the Welder Wildlife Refuge will be collected using automatic ISCO samplers as described above. Samples collected at Riesel will be stored by USDA-ARS for transport by Extension or TWRI to the SAML for analysis. Samples collected at the Welder Wildlife Refuge will be transported by TWRI or Extension to the SAML for analysis. A minimum of 150 ml will be collected by automatic samplers, poured into sterile plastic bottles and stored at 4^{o}C. Edge-of-field samples must be removed from automated samplers and placed in refrigeration within 8 hours of the start of a runoff event, that is, from the first automatically collected stormwater sample. These samples must be transported to SAML, filtered, and placed in the incubator within 48 hours of retrieval from the automated samplers. Samples must be stored at 4oC until processed by SAML. In the event samples can not be processed and incubated within 48 hours, samples will neither be analyzed nor reported.

Processes to Prevent Cross Contamination

To prevent cross-contamination, water samples will be collected directly into sample containers. Field QC samples as discussed in Section B5 are collected to verify that cross-contamination has not occurred.

GPS Tracking of Cattle

Each quarter throughout the project, cattle at the Clear Fork of Plum Creek cooperating ranch will be collared with Lotek® GPS 3300LR collars. Cattle movement will be tracked for 2-3 weeks and then the collars removed. The same cattle will be used each time. At a 5 minute fixed schedule, up to 6,624 locations will be recorded by each collar each quarter.

Streambank Stability Measurements

Permanent cross-sections will be established on the Clear Fork of Plum Creek cooperating ranch on a semi-annual basis using a laser level to allow evaluation in changes to streambank stability as a result of BMP implementation.

Documentation of Field Sampling Activities

Field sampling activities are documented in field notebooks. Records of bacteria analyses are part of the field data record. For all visits, station ID, location, sampling time, sampling date, sampling depth, and sample collector's name/signature are recorded. Values for all measured field parameters are also recorded. Detailed observational data are recorded including water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, missing parameters (items that were to have been sampled that day, but weren't), and days since last significant rainfall.

Recording Data

For the purposes of this section and subsequent sections, all field and laboratory personnel follow the basic rules for recording information as documented below:

- Legible writing in indelible, waterproof ink with no modifications, write-overs or cross-outs;
- Correction of errors with a single line followed by an initial and date;
- Close-outs on incomplete pages with an initialed and dated diagonal line.

Deviations from Sampling Method Requirements or Sample Design, and Corrective Action

Examples of deviations from sampling method requirements or sample design include but are not limited to such things as inadequate sample volume due to spillage or container leaks, failure to preserve samples appropriately, contamination of a sample bottle during collection, storage temperature and holding time exceedance, sampling at the wrong site, etc. Any deviations will invalidate resulting data and may require corrective action. Corrective action may include for samples to be discarded and re-collected. It is the responsibility of the TWRI QAO to ensure that the actions and resolutions to the problems are documented and that records are maintained in accordance with this QAPP. In addition, these actions and resolutions will be conveyed to the TSSWCB Project Manager both verbally and in writing in the project progress reports and by completion of a corrective action report (CAR).

Corrective Action Reports (CARs) document: root cause(s); programmatic impact(s); specific corrective action(s) to address any deviations; action(s) to prevent recurrence; individual(s) responsible for each action; the timetable for completion of each action; and the means by which completion of each corrective action will be documented. CARs will be included with project progress reports. In addition, significant conditions (i.e., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported to the TSSWCB immediately both verbally and in writing.

B3 SAMPLE HANDLING AND CUSTODY

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. The chain-of-custody (COC) form is used to document sample handling during transfer from the field to the laboratory. The sample number, location, date, changes in possession and other pertinent data will be recorded in indelible ink on the COC. The sample collector will sign the COC and transport it with the sample to the laboratory. At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. In the instance that the field sample collector and laboratory sample processor are one in the same, a field-to-lab COC will be unnecessary. A copy of a blank COC form used on this project is included as Appendix B.

Sample Labeling

Samples will be labeled on the container with an indelible, waterproof marker. Label information will include site identification, date, sampler's initials, and time of sampling. The COC form will accompany all sets of sample containers.

Sample Handling

Following collection, samples will be placed on ice in an insulated cooler for transport to the laboratory. At the laboratory, samples will be placed in a refrigerated cooler dedicated to sample storage. The Laboratory Director has the responsibility to ensure that holding times are met with water samples. The holding time is documented on the COC. Any problem will be documented with a CAR.

Failures in Chain-of-Custody and Corrective Action

All failures associated with chain-of-custody procedures as described in this QAPP are immediately reported to the TWRI PM and TWRI QAO. These include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The TWRI PM and QAO will determine if the procedural violation may have compromised the validity of the resulting data. Any failures that have reasonable potential to compromise data validity will invalidate data and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB Project Manager in the project progress report. Corrective action reports will be prepared by the TWRI QAO and submitted to the TSSWCB Project Manager along with project progress report.

B4 ANALYTICAL METHODS

The analytical methods are listed in Table A7.1 of Section A7. *E. coli* in water samples will be isolated and enumerated by SAML personnel using modified mTEC agar, EPA Method 1603 [EPA/821/R-02/023. September 2002. *Escherichia coli* in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia coli* (modified m-TEC) Agar]. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate. The modified medium contains a chromogen, 5-bromo-6-chloro-3-indolyl-ß-D-glucuronide, which is catabolized to glucuronic acid and a red- or magenta-colored compound by *E. coli* that produce the enzyme ß-D-glucuronidase.

Enterococci in water samples will be isolated and enumerated by SAML personnel using mEI agar, EPA Method 1600 [EPA/821-R-02-022. September 2002. *Enterococci* in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-ß-D-Glucoside Agar (MEI)]. The 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. Following filtration, the membrane containing the bacterial cells is placed on a selective medium, mEI agar, and incubated for 24 h at 41°C. All colonies (regardless of color) with a blue halo are recorded as *enterococci* colonies. Magnification and a small fluorescent lamp are used for counting to give maximum visibility of colonies

As outlined in Appendix D, 100 ml water samples will be collected and filtered for analysis of *Bacteroides.* DNA will be extracted from the filters using El Paso Research and Extension Center (EP AREC) SOPs. *Bacteriodes* will then be analyzed using real-time PCR assays performed using published methods [Layton, A. L. McKay, et al. (2006). "Development of *Bacteroides* 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water." Appl Environ Microbiol 72(6): 4214-4224]. Finally, concentrations are calculated from standard curves.

All laboratory sampling areas and equipment will be sterilized with at least one or in any combination of the following methods--ethyl alcohol, bleach, UV light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal.

Table B4.1. Laboratory Analytical Methods

Parameter	Method	Equipment Used
Escherichia coli	EPA 1603	Incubator, filtering apparatus
Enterococci	EPA 1600	Incubator, filtering apparatus
Bacteroides	Extraction = EP AREC SOP PCR = Layton et al. 2006	Corbett Rotor-Gene 6000 real-time PCR Eppendorf Mastercycler ep realplex real-time PCR
Turbidity	EPA 170.1	La Motte® Model 2008 Turbidity Meter

EPA = Methods for Chemical Analysis of Water and Wastes, March 1983

Failures in Measurement Systems and Corrective Actions

Failures in field and laboratory measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, quality control samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the Soil and Aquatic Microbiology Laboratory Director, who will make the determination in coordination with the TWRI QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB as part of this project. The nature and disposition of the problem is reported on the data report. The TWRI QAO will include this information in the CAR and submit with the Progress Report which is sent to the TSSWCB Project Manager.

B5 QUALITY CONTROL

Table A.7-1 in Section A7 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the Project Leader to verify that the data are representative. The Project Leader also has the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of CARs (Appendix A). Laboratory audits, sampling site audits, and quality assurance of field sampling methods will be conducted by the TSSWCB QAO or their designee.

Laboratory Blanks (or Field Blanks)

Laboratory blanks consist of 100-ml aliquots of sterile distilled water that are processed in the same manner as a field sample, at the beginning and the end of a sample set. They are used to assess the sterilization techniques employed throughout the sample process. Laboratory blanks will be included at the beginning and the end of the sample set for each sampling event. The analysis of laboratory blanks should yield a value of no colonies detected. For *Bacteroides* PCR, a laboratory blank will be analyzed with each batch of samples to ensure no cross-contamination occurs during sample processing. In addition, no template negative controls will be analyzed for each batch of PCR samples.

Positive Control

The Soil and Aquatic Microbiology Lab will analyze a positive control for each batch of *Bacteroides* PCR samples.

Laboratory Duplicate

Laboratory duplicates are used to assess precision. A laboratory duplicate is prepared by splitting aliquots of a single sample (or a matrix spike or a laboratory control standard) in the laboratory. Both samples are carried through the entire preparation and analytical process. Laboratory duplicates are run at a rate of one per batch. Acceptability criteria are outlined in Table A7.1 of Section A7.

Precision is calculated by the relative percent difference (RPD) of duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X_1 and X_2, the RPD is calculated from the following equation:

$$\mathbf{RPD} = \frac{(X_1 - X_2) \times 100}{(X_1 + X_2) \div 2}$$

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the laboratory. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair.

Performance limits and control charts are used to determine the acceptability of duplicate analyses. Precision limits for bacteriological analyses are defined in Table A7.1 and applies to samples with concentrations >10 org/100 ml.

Failures in Quality Control and Corrective Action

Notations of blank contamination will be noted in quarterly reports and the final report. Corrective action will involve identification of the possible cause (where possible) of the contamination failure. Any failure that has potential to compromise data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB in the quarterly progress report. The CAR's will be maintained by the Project Leader and the TSSWCB PM.

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

To minimize downtime of all measurement systems, spare parts for field and laboratory equipment will be kept in the laboratory, and all field measurement and sampling equipment, in addition to all laboratory equipment, must be maintained in a working condition. All field and laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in Standard Methods for the Examination of Water and Wastewater, 20th Edition. Maintenance and inspection logs will be kept on each piece of laboratory equipment and general maintenance checklists will be filled out for field sampling equipment, by the field technician, prior to each sampling event.

Records of all tests, inspections, and maintenance will be maintained and log sheets kept showing time, date, and analyst signature. These records will be available for inspection by the TSSWCB.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the quarterly report. The CARs will be maintained by the Project Leader and the TSSWCB PM.

Table B6.1. Equipment Inspection and Maintenance Requirements

Equipment	Relevant Testing, Inspection and Maintenance Requirement
Turbidity meter	SM 2130B
Thermometers	SM 9020 B 3.a
Real-Time PCR Thermocyclers	Product Owner's Manual
Water deionization units	SM 9020 B 3.d
Media dispensing apparatus	SM 9020 B 3.f
Autoclaves	SM 9020 B 3.h
Refrigerator	SM 9020 B 3.i
Ultra Low Freezer	SM 9020 B 3.j
Membrane filter equipment	SM 9020 B 3.k
Ultraviolet sterilization lamps	SM 9020 B 3.l
Biological safety cabinet	SM 9020 B 3.m
Incubators	SM 9020 B 3.o
Glassware and plastic ware	SM 9020 B 4.a
Utensils and containers	SM 9020 B 4.b
Dilution water bottles	SM 9020 B 4.c

B7 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All instruments or devices used in obtaining environmental data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. The instruments requiring calibration are listed below in Table B7.1.

All calibration procedures will meet the requirements specified in the EPA approved methods of analysis. The frequency of calibration as well as specific instructions applicable to the analytical methods recommended by the equipment manufacturer will be followed. All information concerning calibration will be recorded in a calibration logbook by the person performing the calibration and will be accessible for verification during either a laboratory or field audit.

All instruments or devices used in obtaining environmental data will be used according to appropriate laboratory or field practices. Written copies of SOPs are available for review upon request.

Standards used for instrument or method calibrations shall be of known purity and be National Institute of Standards and Technology (NIST) traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society (ACS) or reagent grade quality, or of the best attainable grade. All certified standards will be maintained traceable with certificates on file in the laboratory. Dilutions from all standards will be recorded in the standards log book and given unique identification numbers. The date, analyst initials, stock sources with lot number and manufacturer, and how dilutions were prepared will also be recorded in the standards log book.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the quarterly report. The CARs will be maintained by the Project Leader and the TSSWCB PM.

Table B7.1. Instrument Calibration Requirements

Equipment	Relevant Calibration Requirement
Turbidity meter	Product Owner's Manual
ISCO Bubble Flow Meter	Product Owner's Manual
ISCO Rain Gauge	Product Owner's Manual
Corbett Rotor-Gene 6000 real-time PCR system	Product Owner's Manual
Eppendorf Mastercycler ep realplex real-time PCR	Product Owner's Manual

B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All standards, reagents, media, plates, filters, and other consumable supplies are purchased from manufacturers with performance guarantees, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality, initialed by staff member and marked with receipt date. Volumetric glassware is inspected to ensure class "A" classification, where required. Media will be checked as described in quality control procedures. All supplies will be stored as per manufacturer labeling and discarded past expiration date. In general, supplies for microbiological analysis are received pre-sterilized, used as received, and not re-used.

B9 NON-DIRECT MEASUREMENTS

Water quality determinations at sampling sites will be based upon data collected during the time frame of this project. However, runoff data from small watersheds collected from Riesel by the USDA-ARS, under an approved QAPP, and USGS flow gage data from Plum Creek at Lockhart and Luling will be used as supplemental information to meet data quality objectives (see Section A7).

B10 DATA MANAGEMENT

Field Collection and Management of Routine Samples

Field staff will visit Clear Fork of Plum Creek sampling sites twice a month (on the 1st and 3rd Thursdays of the month) to collect grab water samples and measure field water quality parameters. Field staff will visit Riesel and Welder sites immediately following rainfall events to collect samples and download flow and rainfall data. In addition, these sites will be visited at least monthly to maintain equipment. Site identification, date, time, personnel, water depth, measurements of field parameters, and any comment concerning weather or conditions at the site are noted in the field notebook. A field notebook is filled out in the field for each site visit. If no flow is observed at a site, samples will not be collected but information about the site visit will be recorded in the field notebook.

Samples collected at the site will be labeled and placed in an iced, insulated chest for transportation to the laboratory. A COC form will be used if the collecting technician is in fact not the same person receiving samples into the lab. Site name, time of collection, comments, and other pertinent data are copied from the field notebook to the COC. The COC and accompanying sample bags/bottles are submitted to laboratory analyst, with relinquishing and receiving personnel both signing and dating the COC. All samples transported or mailed to the Soil and Aquatic Microbiology Lab will be accompanied by COC sheets filled out by the field technician.

All COC, field observations, and bacteriological data will be manually entered into an electronic spreadsheet. The electronic spreadsheet will be created in Microsoft Excel software on an IBM-compatible microcomputer with a Windows XP Operating System. The project spreadsheet will be maintained on the computer's hard drive, which is also simultaneously saved in a network folder. All pertinent data files will be backed up monthly on an external hard drive. Current data files will be backed up on an external hard drive monthly and stored in separate area away from the computer.

Original data recorded on paper files will be stored for at least five years. Electronic data files will be archived to CD after approximately the end of the project, and then stored with the paper files for the remaining 4 years.

Laboratory Data

All field samples will be logged upon receipt, COC's (if applicable) will be checked for number of samples, proper and exact I.D. number, signatures, dates, and type of analysis specified. The field technician will be notified if any discrepancy is found and proper corrections made. All samples will be stored at 4ºC until analysis. Bacteriological samples will be given a unique identification number and logged into an electronic spreadsheet. Enumerated bacteriological data will be manually entered into the spreadsheet for electronic storage. The electronic spreadsheet will be created in Microsoft excel software on an IBM-compatible microcomputer with the Windows XP Operating System The project spreadsheet will be maintained on the computer's hard drive, which is also simultaneously saved in an external network folder. All pertinent data files will be backed up monthly on an external hard drive.

Current data files will be backed up on an external hard drive monthly and stored in a separate area away from the computer. At least 10% of all data manually entered in the database will be reviewed for accuracy by the TWRI PM to ensure that there are no transcription errors. Hard copies of data will be printed and housed in the laboratory for a period of five years. Any COC's and bacteriological records related to QA/QC of bacteriological procedures will be housed at the Soil and Aquatic Microbiology Lab.

Data Validation

Following review of laboratory data, any data entry that is not representative of environmental conditions, because it was generated through poor field or laboratory practices, will not be submitted to the TSSWCB. This determination will be made by the Project Leader, Project Co-Leader, TSSWCB QAO, and other personnel having direct experience with the data collection effort. This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the checks specified in Table D2.1.

Data Dissemination

At the conclusion of the project, the Project Leader will provide a copy of the complete project electronic spreadsheet via recordable CD media to the TSSWCB PM, along with the final report. The TSSWCB may elect to take possession of all project records. However, summaries of the data will be presented in the final project report.

C1 ASSESSMENTS AND RESPONSE ACTIONS

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

Table C1.1 Assessments and Response Actions

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TWRI Project Manager	Monitoring of project status and records to ensure requirements are being fulfilled. Monitoring and review of laboratory performance and data quality	Report to TSSWCB in Quarterly Report. Ensure project requirements are being fulfilled.
Laboratory Inspections	Dates to be determined by TSSWCB QAO	TSSWCB QAO	Analytical and quality control procedures employed at laboratory	30 days to respond in writing to TSSWCB to address corrective actions
Monitoring Systems Audit	Dates to be determined by TSSWCB	TSSWCB QAO	Field sampling, handling and measurement; facility review; and data management as they relate to project	30 days to respond in writing to TSSWCB to address corrective actions

Corrective Action

The TWRI Project Leader is responsible for implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the TSSWCB Project Manager and TWRI QAO. Corrective action documentation will be submitted to the TSSWCB Project Manager with the progress report.

If audit findings and corrective actions cannot be resolved, then the authority and responsibility for terminating work is specified in agreements or contracts between participating organizations.

C2 REPORTS TO MANAGEMENT

Quarterly progress reports will be generated by TWRI personnel and will note activities conducted in connection with the water quality monitoring program, items or areas identified as potential problems, and any variation or supplement to the QAPP. The CARs forms will be utilized when necessary (Appendix A) and will be maintained in an accessible location for reference at TWRI. The CARs that result in changes or variations from the QAPP will be made known to pertinent project personnel, documented in an update or amendment to the QAPP and distributed to personnel listed in Section A3. Following any audit performed by the TWRI, a report of findings, recommendations and responses are sent to the TSSWCB Project Manager in the quarterly/monthly progress report.

Field measurements and all sampling for the project will be done according to the QAPP. However, if the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data will be identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problems. The CARs will be filled out to document the problems and the remedial action taken.

Laboratory data reports contain the results of all analyses, as well as specified QC measures listed in section B5. This information is reviewed by the TWRI QAO and compared to the pre-specified acceptance criteria to determine acceptability of data. This information is available for inspection by the TSSWCB.

D1 DATA REVIEW, VERIFICATION AND VALIDATION

All data obtained from field and laboratory measurements will be reviewed and verified for conformance to project requirements, and then validated against the data quality objectives which are listed in Section A7. Only those data which are supported by appropriate quality control data and meet the data quality objectives defined for this project will be considered acceptable. This data will be submitted to the TSSWCB.

The procedures for verification and validation of data are described in Section D2, below. The Extension Project Co-Lead is responsible for ensuring that field data are properly reviewed and verified for integrity. The Soil and Aquatic Microbiology Laboratory Director is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The TWRI PM will be responsible for ensuring that all data are properly reviewed and verified, validated, and submitted in the required format as described by the TSSWCB Project Manager. Finally, the TWRI PM is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

D2 VERIFICATION AND VALIDATION METHODS

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7. The staff and management of the respective field, laboratory, and data management tasks are responsible for the integrity, validation and verification of the data each task generates or handles throughout each process. The field and laboratory tasks ensure the verification of raw data, electronically generated data, and data on chain-of-custody forms and hard copy output from instruments.

Verification, validation and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified (listed by task in Table D2.1) are evaluated against project specifications (Section A7) and are checked for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TWRI Project Lead to establish the appropriate course of action, or the data associated with the issue are rejected.

The TWRI PM and QAO are responsible for validating that the verified data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the data quality objectives of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data for anomalies. The TWRI PM may designate other experienced water quality experts familiar with the water bodies under investigation to perform this evaluation. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data, before data validation can be completed.

A second element of the validation process is consideration of any findings identified during the monitoring systems audit conducted by the TWRI QAO or TSSWCB QAO assigned to the project. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the TWRI PM and QAO validate that the data meet the data quality objectives of the project and are suitable for reporting to the TSSWCB.

Table D2.1. Data Verification Procedures

Data to be Verified	Project Co-Lead	SAML Director	TSSWCB PM/QAO
Collection and analysis techniques consistent with SOPs and QAPP	X	X	X
QC samples collected for all parameters as prescribed in the QAPP	X		X
Field documentation complete	X		X
Instrument calibration data complete	X	X	X
Bacteriological records complete		X	X
Sample documentation complete	X	X	X
Sample identifications	X	X	X
Chain of custody complete/acceptable	X	X	X
Sample preservation and handling	X	X	X
Holding times	X	X	X
QC samples analyzed at required frequencies		X	X
QC samples within acceptance limits		X	X
Instrument readings/printouts	X	X	X
Calculations	X	X	X
Laboratory data verification for integrity, precision, accuracy, and validation		X	X
Laboratory data reports		X	X
Data entered in required format	X	X	X
Site ID number assigned			X
Absence of transcription error	X	X	X
Reasonableness of data	X	X	X
Electronic submittal errors	X	X	X
Sampling and analytical data gaps	X	X	X

D3 RECONCILIATION WITH USER REQUIREMENTS

Extension education programs are designed to target specific audiences and to deliver current, unbiased, science-based information and technology. The objective of the monitoring conducted under this QAPP is to provide the LONE STAR HEALTHY STREAMS Extension education program with unbiased, science-based, quality assured data on the effectiveness of measures for reducing bacteria contamination of streams from grazing lands. No other decisions will be made by the project team based on the data collected.

These data, and data collected by other organizations, may however be subsequently analyzed and used for model development. Thus, data which do not meet requirements will not be submitted to the TSSWCB nor will be considered appropriate for any of the uses noted above.

APPENDIX A. CORRECTIVE ACTION REPORT

Corrective Action Report

CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

State the nature of the problem, nonconformance, or out-of-control situation:

_____
_____
_____
_____
_____

Possible causes:

_____
_____
_____

Recommended corrective action:

_____
_____
_____

CAR routed to:_____

Received by:_____

Corrective Actions taken:

_____
_____
_____
_____

Has problem been corrected? YES NO

Immediate Supervisor:_____

Project Leader:_____

Quality Assurance Officer:_____

APPENDIX B. CHAIN-OF-CUSTODY FORM

TEXAS A&M UNIVERSITY
SOIL AND AQUATIC MICROBIOLOGY LAB
CHAIN OF CUSTODY RECORD

Project Name:					# of containers	Analyses Required											
Station ID	Date	Time (24hr)	Matrix	Description													Sample ID

Relinquished by: (Signature)	Date:	Time:	Received by: (Signature)	Date:	Time:	Laboratory remarks:
Relinquished by: (Signature)	Date:	Time:	Received by: (Signature)	Date:	Time:	Lab log #
Relinquished by: (Signature)	**Date:**	**Time:**	**Received for lab by: (Signature)**	**Date:**	**Time:**	**Laboratory Name:** SAML

APPENDIX C.
BACTERIOLOGICAL DATA LOG SHEET

Bacteriological Data Log Sheet-Membrane Filter

Sample Location	Sample Date	Sampler Initials	Time				Volume Filtered	Colony Count			Temperature		Analyst Initials	Turbidity	Comments
			Collected	Filtered Incubator #1	Incubator #2	Counted		Colony Count X	100 mL vol. filtered	#/100 mL	Initial ©	Final ©			

Flow depth
Estimated flow

20 NTU Turbidity Standard

APPENDIX D. *Bacteroides* PCR

Bacteroides PCR

Preprocessing of Water Samples

1. Within six hours of sample collection, water samples (100 ml) are filtered through 0.2 µm pore size Supor-200 filters (VWR cat # 28147-979). Discard filtrate and place the filter into a pre-labeled sterile 15 ml tube (VWR cat# 21008-103) using ethanol-flamed forceps and aseptic technique. If 100 ml of water cannot be filtered, record the volume filtered on the 15 ml tube and COC.

2. Add 500 µl of guanidine isothiocyanate (GITC) lysis buffer to each 15 ml tube with filter.

   100 ml of GITC lysis buffer
   50 ml reagent grade (deionized) water
   59.08 g GITC (VWR # 100514-046; 5 M final)
   3.7 g EDTA [pH 8.0] (VWR # VW1474-01; 100 mM final)
   0.5 g Sarkosyl (VWR # 200026-724; 0.5% final)
   Adjust to pH 8.0 with NaOH (approx. 0.4 g of pellets) to dissolve EDTA and heat with vigorous stirring to dissolve guanidine
   Bring up to 100 ml total volume with reagent grade (deionized) water
   Autoclave and store at room temp

3. Store samples at -80°C (or -20°C manual defrost freezer, not the standard auto-defrost).

4. DNA will be extracted from the samples and analyzed by *Bacteroides* PCR as described below.

DNA Extraction and PCR

1. DNA is extracted from the water concentrates using QIAamp DNA mini kit. Turn on the slide warmer and set to maximum. Preheat a microfuge tube rack and 0.01X TE buffer pH 8.0 for elution and a 70° C water bath.

2. Add 500 µl of Buffer AL to each thawed tube and vigorously agitate for 1 min using a wrist action shaker.

3. Incubate in a 70° C water bath for 10 minutes.

4. Transfer lysate to a 2.0 ml microfuge tube.

5. Add 500 µl of 100% ethanol and pulse vortex mix for 15 sec. Quick spin to remove droplets from cap.

6. Transfer half of the sample lysate (600 to 750 µl) to a labeled QIAamp column placed in a Qiagen collection tube. Microfuge at 14K rpm, with brake, for 1 minute. If necessary, at each step wipe off any buffer from outside of column with a lab tissue before placing into a new collection tube.

7. Place column in a new collection tube and repeat Step 6 with the remaining sample.

8. Place column in new collection tube and add 500 µl of AW1 wash buffer. Centrifuge as above and place column in a new collection tube.

9. Add 500 µl of AW2 wash buffer and centrifuge as above, then repeat once more. Place column in a clean collection tube and centrifuge as above to remove all traces of AW2 buffer.

10. Place in a clean collection tube in the heated rack on the slide warmer. Add 100 µl of 70 to 80 °C 0.01X TE buffer pH 8.0 and let incubate at 70 to 80 °C for 5 minutes with columns capped.

11. Immediately centrifuge at 14K rpm for 3 minutes and transfer the filtrate containing the eluted DNA to a labeled 0.65 ml tube. Store at -80 °C until analyzed by PCR. Keep the remainder of the unused aliquot of 0.01X TE to use as a no template control for the PCR.

Bacteroides Real-time PCR Assay

1. Real-time PCR assays are performed according to published methods [Layton, A. L. McKay, et al. (2006). "Development of Bacteroides 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water." Appl Environ Microbiol 72(6): 4214-4224] using QuantiTect PCR mix (QIAGEN, Valencia, CA), with 15 pmol of the primer and 5 pmol of the probe (Oligonucleotide primers and 6-carboxyfluorescein (FAM)-BHQ probes from Biosearch Technologies).

2. PCR assays are run with two different sample types:
   - Plasmid DNA containing 16S rRNA genes from *Bacteroides* are run as standards using 10-fold dilutions of the plasmid ranging from 2.5 x 10^7 to 25 copies per PCR.
   - 2.5-µl of DNA extract from water samples in 25-µl PCRs containing QuantiTect master mix and primers and probes.

3. PCR amplification protocols consist of:
   - 50°C for 2 min
   - 95°C for 10 min
   - Up to 50 cycles of 95°C for 30 s and 57°C (BoBac assay) or 60°C (AllBac and HuBac assays) for 45 s

4. PCR amplification and detection of the fluorescent signal is performed using the Eppendorf ® Mastercycler® ep realplex Real-Time PCR system (Eppendorf, Hamburg, Germany) or Corbett Rotor-Gene 6000 real-time PCR system (Corbett Life Science, Sydney, Australia).

5. The threshold cycle (C_T) value for all measurements is determined as the cycle at which fluorescence reaches 5 standard deviations above the background, averaged over 5 cycles collected within the first 15 cycles of PCR amplification.

   For all PCR runs, standards, negative controls (no DNA), and samples are run in triplicate.

6. Concentrations are calculated from standard curves based on the log transformation of known concentrations versus the threshold cycle. Linear correlations are determined using Microsoft Excel.