State General Revenue
Nonpoint Source Grant Program

Evaluation and Demonstration of BMPs for Cattle on Grazing Lands for the Lone Star Healthy Streams Program

TSSWCB Project Number 10-52

Revision #1

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

prepared by

Texas AgriLife Research
Texas Water Resources Institute

Effective Period: Upon TSSWCB Approval through July 2012
(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 *Evaluation and Demonstration of BMPs for Cattle on Grazing Lands for the Lone Star Healthy Streams Program.*

Texas State Soil and Water Conservation Board (TSSWCB)

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

Signature:_____ Date:_____

Name: Pamela Casebolt
Title: TSSWCB Quality Assurance Officer (QAO)

Signature:_____ Date:_____

Texas AgriLife Research, Texas Water Resources Institute (TWRI)

Name: Kevin Wagner
Title: TWRI Associate Director; Project Lead / Project Manager (PM)

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

Signature:_____ Date:_____

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; Project Co-Lead

Signature:_____ Date:_____

A2 TABLE OF CONTENTS

A3 DISTRIBUTION LIST

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

Texas State Soil and Water Conservation Board (TSSWCB)
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Name: Terry Gentry
Tilte: SAML Director, Project Co-Lead

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
CIG	Conservation Innovation Grant
COC	Chain of Custody
CWA	Clean Water Act
DOC	Demonstration of Capability
EMC	Event Mean Concentration
EPA	Environmental Protection Agency
ESSM	Ecosystem Science and Management, Texas A&M University
Extension	Texas AgriLife Extension Service
GLCI	Grazing Lands Conservation Initiative
GPS	Global Positioning System
GRP	Grassland Preserve Program
ICA	Independent Cattlemen's Association
I-Plan	Implementation Plan
LCS	Laboratory Control Sample
LOQ	Limit of Quantitation
LSHS	Lone Star Healthy Streams
NIST	National Institute of Standards and Technology
NRCS	USDA-Natural Resource Conservation Service
NTU	Nephelometric Turbidity Units
OPR	On-going Precision and Recovery
PBS	Phosphate Buffer Solution
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
RTD	Rapid Transfer Device
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
TCFA	Texas Cattle Feeder's Association
TDA	Texas Department of Agriculture
TFB	Texas Farm Bureau
TMDL	Total Maximum Daily Load
TSCRA	Texas Southwestern Cattle Raiser's Association
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas Water Resources Institute
USDA	United States Department of Agriculture
USGS	United States Geological Survey
WPP	Watershed Protection Plan
WWF	Welder Wildlife Federation
WWR	Welder Wildlife Refuge

A4 PROJECT/TASK ORGANIZATION

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

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.

Pamela Casebolt, 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 AgriLife Research, Texas Water Resources Institute (TWRI)

Kevin Wagner, TWRI Associate Director; Project Lead / Project Manager

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. Also, 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

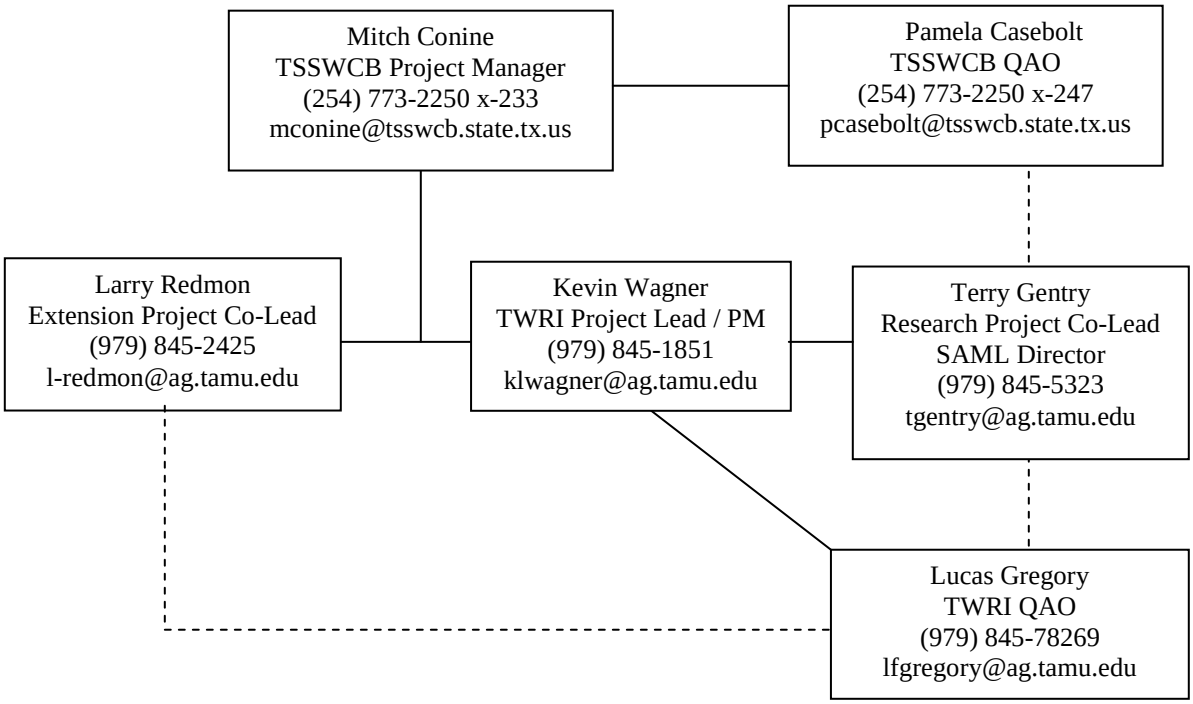
Responsible for verifying that the project is producing data of known and acceptable quality. Responsible for supervising all aspects of the sampling and measurement of surface waters and other parameters in the field. Responsible for field staffing and ensuring that staff is appropriately trained.

Texas AgriLife Research

Terry Gentry, SAML Director, Project Co-Lead

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 2008 Texas Water Quality Inventory and 303(d) List, recreation is impaired in 274 waterbody segments and oyster harvest is impaired in another 21 due to bacteria. To address the bacteria impaired waterbodies, Texas is developing and implementing total maximum daily loads (TMDLs), TMDL Implementation Plans (I-Plan), and Watershed Protection Plans (WPPs). One of the primary strategies for reducing bacteria in many of these waterbodies is to provide technical and financial assistance to implement best management practices (BMPs) to reduce bacteria runoff from cattle on grazing lands. Because grazing land is the dominant land use in the state, there is a statewide need for BMPs targeted to this land use and livestock category. However, in order inspire behavior change, evaluations and demonstrations of BMP effectiveness are needed to encourage voluntary implementation of BMPs and participation in federal and state technical and financial assistance programs to reduce the runoff of bacteria which will ultimately lead to improved water quality.

The evaluation of BMPs for cattle on grazing lands was initiated with Grassland Reserve Program (GRP) funds made available by the TSSWCB through the USDA-NRCS Environmental Management of Grazing Lands (TSSWCB Project 06-14), Clean Water Act §319(h) grant funds made available by the TSSWCB through the U.S. Environmental Protection Agency (EPA) Lone Star Healthy Streams (TSSWCB Project 06-5), and Conservation Innovation Grant (CIG) funds provided by USDA-NRCS Bacteria Runoff BMPs for Intensive Beef Cattle Operations. The development of a comprehensive education program founded on the evaluation of BMPs in those projects is being supported with CWA §319(h) grant funds made available by the TSSWCB through EPA Development of a Synergistic, Comprehensive Statewide Lone Star Healthy Streams Program (TSSWCB Project 09-06).

Continued support is needed to advance work to evaluate BMPs and verify their beneficial impacts to provide the scientific backbone of AgriLife Extension educational programs (i.e., Lone Star Healthy Streams). Both continued evaluation of new publications/articles/research and field evaluation and demonstration of BMPs is needed to ensure the most up-to-date and relevant information is available for Texas ranchers, as well as, decision-makers at the TSSWCB, USDA-NRCS and livestock groups in the state. Only through continued demonstration of BMPs, educational programs, and landowner assistance for implementing effective BMPs will significant progress be made to restore water quality across the state.

A6 PROJECT/TASK DESCRIPTION

General Project Description

This project will continue and further the work begun by previous projects as described above. The Lone Star Healthy Streams (LSHS) Project Steering Committee, originally organized through TSSWCB project 06-05, will continue to provide guidance and oversight for this project. This Steering Committee is a partnership of the primary federal and state agencies that interface with beef cattle producers and cattle industry organizations. The Steering Committee is facilitated by TWRI and SCSC and includes ranchers and representatives from the TSSWCB, Soil and Water Conservation Districts (SWCDs), USDA-NRCS, USDA-ARS, TWRI, Texas AgriLife Extension Service, Texas AgriLife Research, Texas Department of Agriculture (TDA), Grazing Lands Conservation Initiative (GLCI), Texas Farm Bureau (TFB), Texas and Southwestern Cattle Raisers Association (TSCRA), Independent Cattlemen's Association of Texas (ICA), Texas Cattle Feeders Association (TCFA), and the Welder Wildlife Foundation(WWF). This LSHS Project Steering Committee will provide input on the evaluation of BMP effectiveness, identification of demonstration sites, modifications to the LSHS curriculum, and general project coordination. This LSHS Project Steering Committee will meet as frequently as needed, likely annually.

SCSC, in coordination with TWRI and SAML, will continue to assess and demonstrate the efficacy and impacts of BMPs identified by the LSHS Project Steering Committee. Because of low rainfall, additional time for evaluation of grazing management and stocking rates/densities is needed. Three grazing treatments will be evaluated – no grazing, moderate grazing, and heavy grazing at the Brazos Bottom, Welder Wildlife Refuge, and Riesel demonstration sites. SAML will continue to analyze the water samples from the grazing management areas for *E. coli* using EPA approved methods. Additionally, levels of *Enterococcus* spp. and fecal coliforms will be assessed at these sites.

SCSC, with assistance from TWRI and ESSM, will evaluate the effectiveness of certain structural BMPs in modifying cattle movement to change fecal deposition patterns and reducing bacteria runoff. BMPs that have been identified as needing evaluation include (1) portable shade facilities, (2) protected stream access points or stream crossing, (3) rip-rap application designed to limit/block cattle access to riparian areas, and (4) additional evaluation of the impacts of alternative water supplies designed to draw cattle away from waterbodies. Evaluation of protected stream access points or stream crossings will be dependent on finding a cooperator where USDA-NRCS is designing and constructing this practice. Effects of these BMPs on cattle behavior and bacteria levels will be evaluated and demonstrated to beef cattle producers. The effect of portable shade facilities on cattle behavior was evaluated at a private ranch in the Plum Creek watershed through TSSWCB project 06-05; however, evaluation of a different configuration of the shade structure is needed. Additionally, at the same private ranch in the Plum Creek watershed, alternative water supplies were evaluated, but little riparian vegetation was present. Thus, additional monitoring will be needed to fully evaluate this practice in an area where there is extensive riparian vegetation. Cooperating ranch(es) will be identified for this demonstration and the other practices. USDA-NRCS will assist with identifying cooperating ranches, especially for protected stream access points or stream crossings. These are engineering-

intensive practices and as such, SCSC will work with USDA-NRCS to identify where such practices are being designed and installed. USDA-NRCS may also assist in identifying ranches for evaluation of alternative water supplies.

SCSC will hire a graduate student to execute the BMP effectiveness studies and other project tasks. SCSC will work closely with SAML, ESSM, TWRI and staff from TSSWCB project 09-06.

TWRI and SCSC will attend and participate in public meetings in order to communicate project goals, activities and accomplishments to affected parties. Such meetings may include the Annual Meeting of Texas SWCD Directors, the TSCRA Annual Convention, the TFB Annual Convention, Clean Rivers Program Basin Steering Committee meetings, and watershed stakeholder meetings for certain TMDLs and WPPs. TWRI and SCSC will develop and disseminate project informational materials, including, flyers, brochures, letters, and news releases. TWRI will continue to host and maintain an internet website http://grazinglands-wq.tamu.edu/ for the dissemination of information.

SCSC will continue to gather information from the growing body of literature on 1) bacterial fate and transport, 2) effects of grazing cattle on bacterial levels in waterbodies, and 3) effect of BMPs designed to minimize grazing cattle impacts on riparian areas and bacterial loading. A compendium of this literature will be posted on the project website.

SCSC, with assistance from TWRI and USDA-ARS and in cooperation with SWCDs and local Extension and USDA-NRCS staff, will conduct at least 1 field day at a demonstration site to highlight the BMP effectiveness studies and promote adoption of BMPs by ranchers.

SCSC, with assistance from TWRI, will develop technical reports, refereed journal articles, Extension Fact Sheets, and other publications, summarizing the results of the demonstrations (grazing management treatments and structural BMP evaluation) and the analysis of the impacts of BMPs on bacteria runoff. Based on the findings of these demonstrations and BMP evaluations, the LSHS program curriculum will be modified and updated to highlight BMP effectiveness studies and promote adoption of BMPs by ranchers.

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	Prepare & submit quarterly reports to TSSWCB & participants	TWRI	06/10	07/12
1.2	Perform accounting functions	TWRI	06/10	07/12
1.3	Conduct quarterly meetings with project participants.	TWRI	06/10	07/12
1.4	Participate in public meetings	TWRI/SCSC	06/10	07/12
1.5	Develop & disseminate project materials	TWRI/SCSC	06/10	07/12
1.6	Host & maintain project website	TWRI	06/10	07/12
1.7	Conduct LSHS Project Steering Committee meetings.	TWRI/SCSC	06/10	07/12
1.8	Develop & submit Final Report	TWRI/SCSC	06/10	07/12
2.1	Develop QAPP	TWRI	06/10	08/10
2.2	QAPP Annual Revision #1	TWRI	06/11	08/11
3.1	Evaluate grazing management	SCSC/TWRI	09/10	07/12
3.2	Evaluate structural BMPs	SCSC/TWRI	09/10	07/12
3.3	Compile literature review	SCSC	06/10	07/12
3.4	Identify cooperator(s) for BMP demonstration	SCSC/TWRI	06/10	08/10
3.5	Assess GPS collar data	TWRI/SCSC	09/10	07/12
3.6	Conduct field day	SCSC/TWRI	09/10	07/12
3.7	Design WQMP monitoring regime	TWRI/ARS/SAML	06/10	07/12
3.8	Transfer results to ARS for incorporation into TBET	TWRI/ARS	06/10	07/12
3.9	Establish NRCS practice standard for Livestock Shade Structures	TWRI/SCSC/NRCS	06/10	07/12
3.10	Develop technical reports, journal articles, fact sheets, etc.	SCSC/TWRI	04/12	07/12

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), two 1.2-ha sites located at the USDA-ARS Grassland Soil and Water Research Laboratory near Riesel (SW-12, W-10), and three 1-ha watershed sites located at the Texas A&M University (TAMU), Department of Animal Science, Beef Cattle Systems Center located west of the TAMU campus on Highway 50, along the banks of the Brazos River between College Station and Snook. On the Welder Wildlife Refuge, WWR-1 will be ungrazed rangeland, WWR-3 will be moderately grazed rangeland, and WWR-2 will be heavily grazed rangeland. At Riesel, SW-12 is an ungrazed native prairie reference site and W-10 is a moderately grazed coastal bermudagrass pasture. At the Beef Cattle Systems Center, BB-1 will be ungrazed irrigated Tifton 85 pasture, BB-2 will be moderately grazed irrigated Tifton 85 pasture, and BB-3 will be heavily grazed irrigated Tifton 85 pasture. Rainfall depth, rainfall intensity, and flow will be measured for each event. Event mean concentrations for *E. coli, Enterococcus* and fecal coliforms will be determined for each runoff event where sufficient sample volume is available.

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]. Fecal coliform will be analyzed by the SAML using the fecal coliform membrane filter procedure [American Public Health Association, American Water Works Association, Water Environment Federation (1999). Standard Methods for the Examination of Water and Wastewater, Method 9222D. Fecal Coliform Membrane Filter Procedure].

The *Bacteroidales* 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 *Bacteroidales* to the total *Bacteroidales* detected for each sample will be estimated. For pre-processing of water samples for *Bacteroidales* 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) 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.

In the previous Lone Star Healthy Streams project (TSSWCB Project Number 06-05), *Bacteroidales* PCR results had a very strong correlation with *E. coli* levels in runoff at one location (Brazos Bottom near College Station) but not as strong of a correlation at the other locations (Welder Wildlife Refuge near Sinton and USDA-ARS at Riesel). Since the standard curve used for the *Bacteroidales* PCR was developed from a cattle fecal sampled collected at the Brazos Bottom, this suggests a potential geographical bias for the *Bacteroidales* PCR. In order to test this, water samples previously collected from Riesel (during the initial Lone Star Healthy Streams project, TSSWCB Project Number 06-05) will be re-analyzed with Riesel-specific *Bacteroidales* PCR standard curves. Additional water samples may be collected and tested, as available.

Structural BMPs will be evaluated over a period of 2 years utilizing GPS collars. Structural BMPs that have been identified as needing evaluation include (1) portable shade facilities/structures, (2) protected stream access points or stream crossing, (3) rip-rap application designed to limit cattle access to riparian areas, and (4) alternative water supplies designed to draw cattle away from waterbodies. Changes in cattle movement will be evaluated using GPS collars. SCSC and ESSM will assess cattle behavior in response to BMPs utilizing Lotek GPS collars to determine the amount of time cattle spend in the stream and riparian areas before and after BMP implementation. TWRI will assist with GPS collar data analysis. Reductions in

bacteria contributions will be calculated based on the reduced time cattle spend in the stream and riparian area. Evaluation of protected stream access points or stream crossings will be dependent on finding a suitable cooperator where USDA-NRCS is designing and constructing this practice. Portable shade and alternative water supplies will be evaluated at the McGregor Research Center. Rip rap will be evaluated at both the Beef Cattle Systems Center and the McGregor Research Center.

A7 QUALITY OBJECTIVES AND CRITERIA

The project objectives are to: (1) reduce bacteria contamination caused by grazing livestock in Texas waterbodies through evaluation and demonstration of BMP effectiveness in reducing bacteria runoff from grazing lands and (2) utilize BMP effectiveness data as the scientific-basis for the Lone Star Healthy Streams (grazing cattle component) education program. Measurement performance specifications to support the project objective are specified in Table A7.1.

Table A7.1. Measurement Performance Specifications

PARAMETER	UNITS	METHOD	LOQ	Precision of Laboratory Duplicates	Bias	Percent Complete
E. coli	*cfu/100 ml*	*EPA 1603*	*10*	*3.27 * ΣRlog/n*	*NA*	*90*
Enterococci	*cfu/100 ml*	*EPA 1600*	*10*	*3.27 * ΣRlog/n*	*NA*	*90*
Fecal coliform	*cfu/100ml*	*SM 9222D*	*10*	*3.27 * ΣRlog/n*	*NA*	*90*
Bacteroidales PCR	*orgs/100ml*	*Extraction = EP AREC SOP PCR = Layton et al. 2006*	*NA*	*100% agreement*	*90% correct*	*90*

Ambient Water Reporting Limits And Laboratory Reporting Limits

It is not the objective of this project to evaluate ambient water quality conditions; thus, ambient water reporting limits (AWRLs) are not applicable and are not needed to yield data acceptable to meet project objectives. The limit of quantitation (LOQ) [formerly known as the reporting limit (RL)] is the minimum level concentration, or quantity of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence. The LOQ for target analytes are set forth in Table A7.1. For indicator bacteria analysis in water, the LOQ is a result of the sample volume filtered. Sample volumes routinely filtered for indicator bacteria in runoff are 10, 1, 0.1, and 0.01 ml. Thus, the LOQ for indicator bacteria for runoff water quality samples analyzed for this project is 10 cfu/100 ml.

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. Laboratory precision is assessed by comparing sample/duplicate pairs. 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 prepared with verified and known amounts of all target analytes in the sample matrix and by calculating percent recovery. For *E. coli* in water, SAML will routinely process and analyze BioBall^{TM} spiked PBS samples.

SAML will analyze one ongoing precision and recovery (OPR) sample for every batch of runoff samples. Results will be compared against the measurement performance specifications in Table A7.1 and used during evaluation of analytical performance.

An additional element of bias is the absence of contamination. This is determined through the analysis of blank samples processed in a manner identical to the sample. OPR samples must be accompanied by an acceptable method blank and processed according to method specifications. Requirements for blank samples are further discussed in Section B5.

Representativeness

Representativeness of each runoff event will be ensured by collection of flow-weighted samples throughout the entire hydrograph of each runoff event. Additionally, representativeness will be ensured by the analysis of runoff from 8 different sites representing a variety of land uses (pasture, native prairie, and rangeland), stocking rates, and grazing management (ungrazed, moderate grazed, and heavy grazed). Finally, representativeness will be measured with the completion of sample collection in accordance with the approved QAPP.

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 data in standard units, by using accepted rules for significant figures, by reporting data in a standard format, and by reporting all data (including QC data) for evaluation by others.

Completeness

The completeness of the data is basically a relationship of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to accidents, 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% data completion is achieved.

A8 SPECIAL TRAINING/CERTIFICATION

No special certifications are required. However, new field and lab personnel will receive training in proper sampling and sample analysis. Before actual sampling or analysis occurs, they will demonstrate to the project co-lead responsible for the given sampling or analysis task (as described in Section A4) their ability to properly perform field sampling or analysis procedures. Finally, SAML is NELAC™-accredited for enumerating *E. coli* in both non-potable and drinking water using USEPA Method 1603. SAML *Personnel, Training, and Data Integrity* requirements are provided in Section 17 of the SAML Quality Manual and *Demonstration of Capability (DOC)* and *On-Going Proficiency* requirements are provided in Sections 19.1 and 19.2, respectively.

A9 DOCUMENTS AND RECORDS

The documents 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
Chain of custody records	SAML	2 years	Paper
Corrective action reports	TWRI/SAML	2 years	Paper/Electronic
Bacteriological data sheet	SAML	2 years	Paper
Laboratory QA manuals and/or SOPs	SAML	5 years	Paper/Electronic
Lab equipment calibration records & maintenance logs	SAML	2 years	Paper
Lab data reports/results	TWRI/SAML	2 years	Paper/Electronic
GPS collar data	TWRI/Extension	2 years	Electronic
Progress reports/final report/data	TWRI	5 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. 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.

B1 SAMPLING PROCESS DESIGN

The goal of the monitoring 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 edge of field bacteria runoff and cattle movement for the purpose of aiding evaluation of BMP effectiveness in reducing bacteria loadings under various scenarios. Best management practices will be evaluated at four or more locations: the Welder Wildlife Refuge located in the Copano Bay watershed, the USDA-ARS Grassland Soil and Water Research Laboratory near Riesel, Texas A&M University (TAMU) Department of Animal Science Beef Cattle Systems Center near College Station, the Department of Animal Science McGregor Research Center, and one or more private ranches, location(s) to be determined. Information gained from this project will be used to educate landowners concerning bacterial impairments and effectiveness of BMPs focused on reducing potential contamination sources.

Evaluation of Grazing Management

The effects of grazing management on bacteria runoff will be evaluated at eight sites, 3 sites located at the Welder Wildlife Refuge near Sinton, 2 sites at the USDA-ARS Grassland Soil and Water Research Laboratory near Riesel, and 3 sites at the Beef Cattle Systems Center near College Station (Table B1.1 and Figures B1.1, B1.2, and B1.3).

Table B1.1. Grazing Management Sample Sites and Grazing Management

Station	Size	Long Description (lat/long)	Grazing Management	SR*
WWR-1	1.0 ha	28° 6'55.97"N / 97°21'20.82"W	Ungrazed Rangeland	NA
WWR-2	1.0 ha	28° 6'51.98"N / 97°21'21.89"W	Heavy Grazed Rangeland	7
WWR-3	1.0 ha	28° 6'52.60"N / 97°21'13.83"W	Moderately Grazed Rangeland	14
SW-12	1.2 ha	31° 28’48”N / 96° 52’59”W	Ungrazed Native Prairie	NA
W-10	8.0 ha	31° 27’12”N / 96° 53’0”W	Moderately Grazed Bermudagrass	5
BB-1	1.0 ha	30° 31'44.3"N / 96°24'58.3"W	Ungrazed Irrigated Tifton 85	NA
BB-2	1.0 ha	30° 31'47.5"N / 96°24'57.7"W	Moderately Grazed Irrigated Tifton 85	2
BB-3	1.0 ha	30° 31'47.7"N / 96°24'57.9"W	Heavy Grazed Irrigated Tifton 85	1

*SR = Approximate stocking rate in acres per animal unit

For each runoff event, *E. coli*, *Enterococci*, fecal coliforms, and flow will be measured (Table B1.2) at each site.

Table B1.2. Grazing Management Monitoring Parameters

Parameter	Status	Reporting Units
E. coli	Critical	cfu per 100 milliliters (cfu/100 ml)
Enterococci	Critical	cfu per 100 milliliters (cfu/100 ml)
Fecal coliform	Critical	cfu per 100 milliliters (cfu/100 ml)
Flow	Critical	cubic feet per second (cfs)

All sites are equipped with berms and v-notch weirs to aid in collection and measurement of runoff. Additionally, at each site an ISCO® bubble flow meter and sampler is installed to measure flow and collect runoff. ISCO® samplers are programmed to collect flow-weighted

composite samples allowing determination of event mean concentrations (EMCs) for *E. coli*, *Enterococci* and fecal coliforms for each rain event.

Figure B1.1. Welder Wildlife Refuge Sites

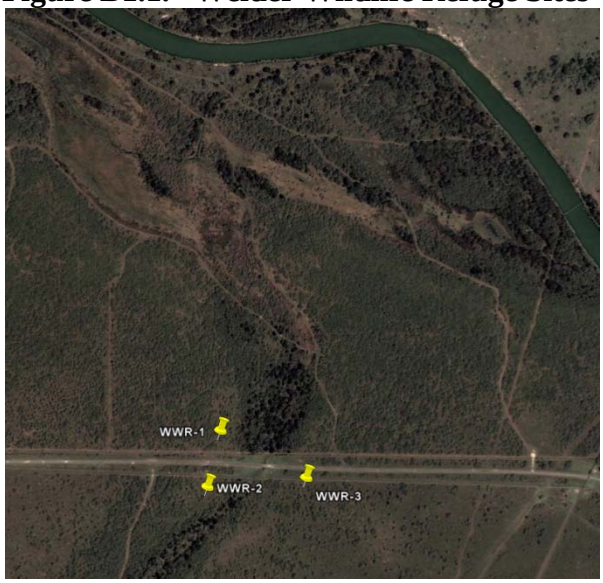


Figure B1.2. USDA-ARS Research Lab at Riesel Sites

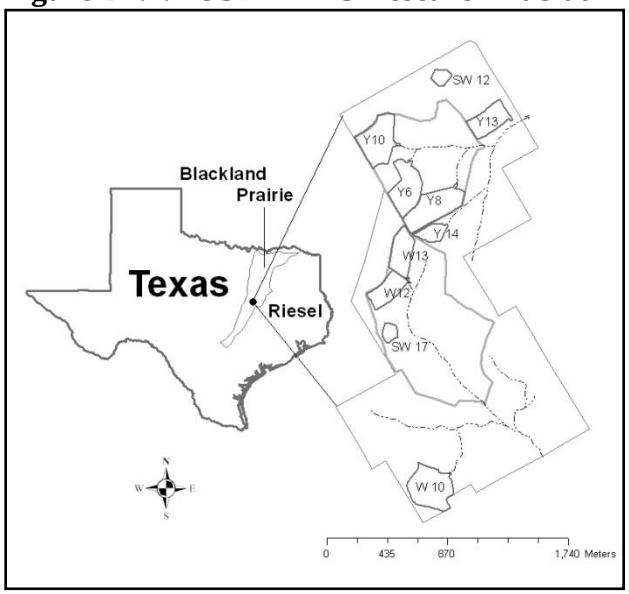
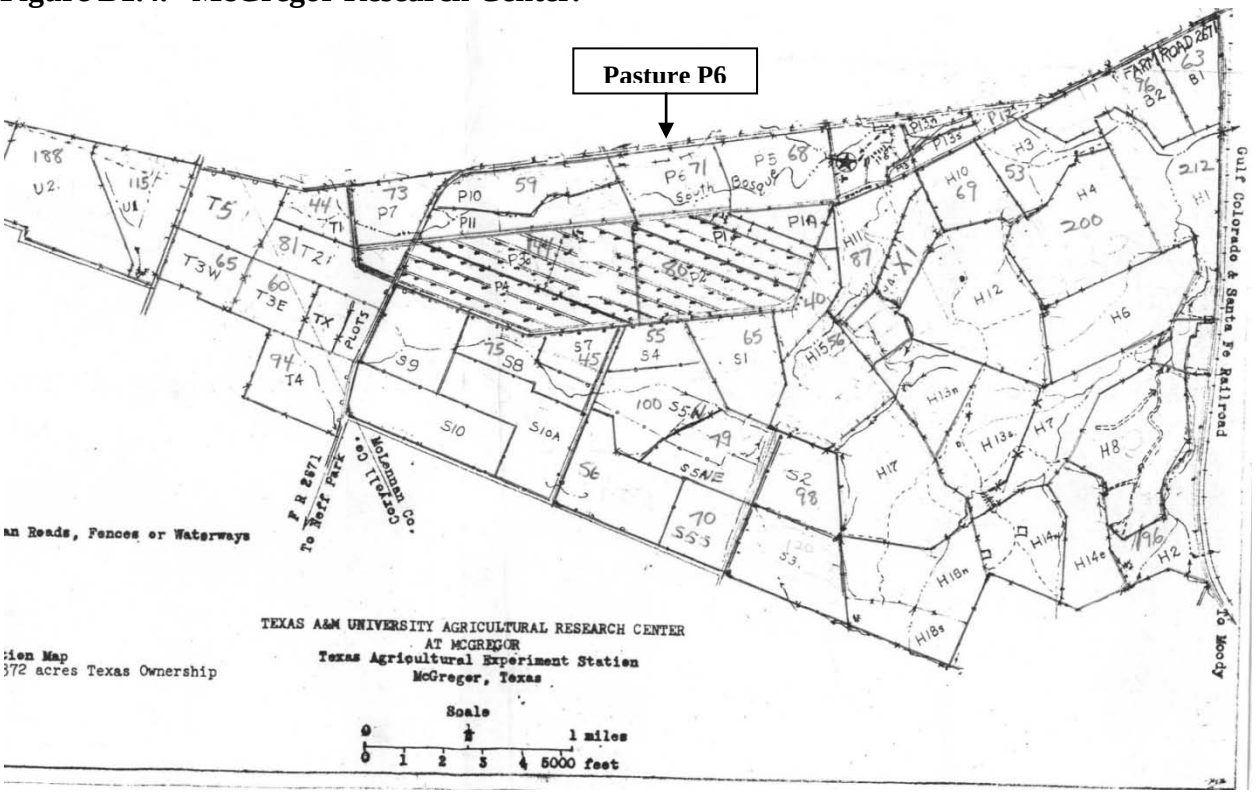


Figure B1.3. Beef Cattle Systems Center Sites

Evaluation of Structural BMPs

The effects of structural BMPs on the percent time cattle spend in and adjacent to streams will be evaluated using GPS collars. Portable shade facilities/structures, rip-rap application designed to limit cattle access to riparian areas, and alternative water supplies designed to draw cattle away from waterbodies will be evaluated at the Texas A&M Animal Science Department McGregor Research Center. Pasture P6 will primarily be used for these studies (Figure B1.4).

Figure B1.4. McGregor Research Center.



This 71 acre pasture is located on the headwaters of the South Bosque River. It has a typical riparian area with little shade outside the riparian area. Alternative water is also already available, but can be turned off and on as needed to assess alternative water. Eight Lotek GPS collars will be used for this evaluation. These collars will be placed on 8 randomly selected cows from a herd size of approximately 20-25 head placed on pasture P6 for the evaluation. Use of AgriLife Research owned cattle will be in accordance with Texas A&M University Animal Use Committee requirements.

Each of the 3 BMPs (rip-rap, alternative water, and shade) will be evaluated at least twice during the project using the following protocol:

- No BMP = Minimum of 3 days
- Transition = Minimum of 2 days
- BMP = Minimum of 3 days

In addition to the monitoring described above, rip-rap will also be evaluated at the Beef Cattle Systems Center near College Station at the grazed sites described in the previous section. No GPS collars will be used for this assessment. Visual observation will be used to assess the effects of various widths of rip-rap on cattle use of a second water trough placed in the grazed pastures. The purpose of this assessment is to determine the appropriate width of the rip rap before application at field scale at the McGregor Research Center.

Finally, protected stream access points or stream crossing will be evaluated at a cooperating ranch where USDA-NRCS is designing and constructing this practice. During the design and construction phase, Lotek GPS collars will be placed on 8 randomly selected cows in the cooperators herd for a period of 21-23 days to evaluate the pre-BMP scenario. Once construction is completed and cattle are accustomed to the BMP, then the GPS collars will be re-applied to evaluate their location for another 21-23 days. This will allow a pre-/post-BMP comparison of percent time cattle spend in and near the stream.

B2 SAMPLING METHODS

Edge of Field Sampling Procedures for Grazing Management Evaluation

Flow-weighted composite edge of field samples from each of the eight sites listed in Table B1.1 will be collected using ISCO® 6712 full-size portable samplers with single bottle configuration into sterile polyethylene 4-gallon round bottles. A minimum of 50 ml will be collected by automatic samplers. For transport to SAML, the samples in the 4-gallon bottles will be thoroughly mixed and a sub-sample transferred to a sterile bacteriological bottles or Whirl-Pak® bag, placed on ice in a cooler, and stored at 4°C until analysis (Table B2.1). 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 Beef Cattle Systems Center and Welder Wildlife Refuge will be transported by Extension or TWRI to the SAML for analysis. This collection of a flow-weighted composite sample will allow calculation of event mean concentrations of bacteria for each rainfall event and determination of total annual loadings. 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 runoff event. Flow data will be downloaded at least monthly using an ISCO® 581 Rapid Transfer Device (RTD).

Table B2.1. Field Sampling and Handling Procedures

Parameter	Matrix	Container	Preservation	Sample Volume	Holding Time
E. coli	Water	Sterile bacteriological bottles / Whirl-Ppak® bags	4°C	12-22 ml	48 hours[1]
Enterococci	Water	Sterile bacteriological bottles / Whirl-Pak ® bags	4°C	12-22 ml	48 hours[1]
Fecal coliform	Water	Sterile bacteriological bottles / Whirl-Pak ® bags	4°C	12-22 ml	48 hours[1]
Bacteroidales	Water	Sterile bacteriological bottles	GITC buffer	100 ml	6 hours[1]
MIN. NEEDED	**Water**	**Sterile bacteriological bottles** / Whirl-Pak ® **bags**	**4°C**	**136-166 ml**	**6 hours**

[1] 6 hours to deliver to laboratory. In the case that this 6-hour holding time is not met, the *E. coli* quantitative count and *Bacteroidales* data will be flagged, though the non-quantitative source identification will still be valid.

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 samples are held below 10°C and not allowed to freeze. Pope reported a majority of sites showed no significant differences in *E. coli* densities between the 0- and 48-hour holding times. Pope also reported, a majority of *E. coli* samples held at 20 and 35°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]. Thus, all samples must be transported to SAML, filtered, and placed in the incubator within 48 hours of retrieval from the automated samplers. The 48 hours begins with the collection time of the first runoff sample from each ISCO®. 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 sterile bacteriological bottles or new Whirl-Pak® bags.

GPS Tracking of Cattle for Evaluation of Structural BMPs

As described in section B1, randomly selected cattle at the McGregor Research Center and cooperating ranch will be collared with Lotek® GPS 3300LR collars. Cattle movement will be tracked for 21-23 days and then the collars removed. Data will be downloaded from the collars by the Extension Range Specialist located at the Texas AgriLife Research and Extension Center at Uvalde and emailed to TWRI and Extension. TWRI and Extension will use ArcView to assess the percent time cattle spend within various distances from the stream. At a 5 minute fixed schedule, up to 6,624 locations will be recorded by each collar each deployment.

Documentation of Field Sampling Activities

Field activities are documented as needed in field notes. For all water samples collected, station ID, sampling date and time, sample type, and sample collector's name/signature are recorded on the sample container and COC.

Recording Data

All field and laboratory personnel follow the basic rules for recording information as follows:

- Legible writing in indelible ink with no modifications, write-overs or cross-outs;
- Correction of errors with a single line followed by an initial and date; and
- 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

Fecal coliform will be analyzed by the SAML using the fecal coliform membrane filter procedure [American Public Health Association, American Water Works Association, Water Environment Federation (1999). Standard Methods for the Examination of Water and Wastewater, Method 9222D. Fecal Coliform Membrane Filter Procedure].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, M-FC medium, and incubated for 24 h at 44.5°C. Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Magnification and a small fluorescent lamp are used for counting to give maximum visibility of colonies

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.

As outlined in Appendix E, 100 ml water samples will be collected and filtered for analysis of *Bacteroidales.* The DNA will be extracted from the filters using El Paso Research and Extension Center (EP AREC) SOPs. In addition, selected samples collected and previously processed during the previous Lone Star Healthy Streams project (TSSWCB Project Number 06-05) will also be tested. Finally, concentrations are calculated from standard curves.

Table B4.1. Laboratory Analytical Methods

Parameter	Method	Equipment Used
E. coli	EPA 1603	Incubator, filtering apparatus
Enterococci	EPA 1600	Incubator, filtering apparatus
Fecal coliform	SM 9222D	Incubator, filtering apparatus
Bacteroidales	Extraction = EP AREC SOP PCR = Layton et al. 2006	PCR

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 and complete the analysis. If the problem is not resolvable, then it is conveyed to the SAML 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 A7.1 lists the required accuracy, precision, and completeness limits for the parameters of interest. Specific requirements are summarized in Table B5.1 and described below.

Table B5.1. Required Quality Control Analyses

Parameter	Matrix	LCS	Lab Dup	Method Blank
E. coli	Water	√	√	√
Enterococci	Water	*NA*	√	√
Fecal Coliform	Air	*NA*	√	√

Laboratory Control Sample (LCS)

An LCS consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system. The LCS is spiked into the sample matrix at a level less than or near the midpoint of the calibration for each analyte. The LCS is carried through the complete preparation and analytical process. LCSs are run at a rate of one per preparation batch for the analysis of *E. coli* in water. Results of LCSs are calculated by percent recovery (%R), which is defined as 100 times the measured concentration, divided by the true concentration of the spiked sample. The following formula is used to calculate percent recovery, where %R is percent recovery; SR is the measured result; and SA is the true result:

$$\%R = SR/SA * 100$$

Measurement performance specifications are used to determine the acceptability of LCS analyses as specified in Table A7.1.

Laboratory Duplicates

One bacteriological duplicate analysis will be performed for each batch of runoff samples. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair. 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 * ΣRlog/n, where Rlog is the difference in the natural log of duplicates for the first 15 positive samples.

Measurement performance specifications are used to determine the acceptability of duplicate analyses as specified in Table A7.1. The specifications for bacteriological duplicates in Table A7.1 apply to samples with concentrations > 10 org./100mL.

Method blank

A method blank is a sample of matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. The method blanks are performed at a rate of once per batch. The method blank is used to document contamination from the analytical process.

A method blank will be run along with all water quality samples and will consist of 100-ml of phosphate buffer solution (PBS) solution processed in the same manner as a field sample. The analysis of laboratory blanks should yield a value of no colonies detected. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the samples (e.g. reprocessing or data qualifying codes). In all cases the corrective action must be documented.

Failures in Quality Control and Corrective Action

Results of the analyses of QC samples (i.e. lab control standards, lab duplicates, and method blanks) will be routinely monitored and evaluated by the SAML Lab Director. The disposition of quality control failures and the nature and disposition of the problem is reported to the TWRI QAO. The TWRI QAO will discuss with the TWRI Project Manager. Corrective action will involve identification of the possible cause (where possible) of the QC failure. Any failure that has potential to compromise data validity will invalidate data, and the sampling event will be repeated if possible. The resolution of the situation will be reported to the TSSWCB via CAR in the quarterly progress report. The CAR's will be maintained by the TWRI QAO and PM.

The definition of and process for handling deficiencies and corrective action are defined in Section C1.

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. 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. Maintenance of the ISCO® automated samplers will be conducted at least monthly and documented on an ISCO® Sampler Maintenance form (Appendix D).

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.

B7 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All instruments or devices used in obtaining environmental data for this project will be calibrated according to and at the frequency recommended by the equipment manufacturer's instructions as each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. In this project, the primary instrument requiring calibration is the ISCO® Bubble Flow Meter. All information concerning calibration of the ISCO® Bubble Flow Meters will be recorded on an ISCO® Sampler Maintenance form (Appendix D) by the person performing the calibration and will be accessible for verification during either a laboratory or field audit.

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.

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

Runoff monitoring and analysis of *E. coli* at Riesel as described throughout this QAPP will be performed under TSSWCB Project 09-05 and its QAPP. However, lab analysis of runoff from Riesel for fecal coliform and *Enterococci* do fall under this QAPP.

Additionally, data previously collected at the 2S Ranch, Beef Cattle Systems Center, Riesel and the Welder Wildlife Refuge following the QAPP for Lone Star Healthy Streams (TSSWCB Project Number 06-05) will also be utilized as supplemental information to meet data quality objectives (see Section A7).

B10 DATA MANAGEMENT

Field Collection and Management of Samples

All field collection will be completed as described in Section B2 of the QAPP. A Chain of Custody is filled out for each sampling event noting the site name, time and date of collection, sample type, comments, sample collector's name, and other pertinent data. Samples collected will be labeled with site identification, date, sampler's initials, and time of sampling and transported to the laboratory as outlined in B3. Finally, the COC and accompanying sample bags/bottles are submitted to SAML, with relinquishing and receiving personnel both signing and dating the COC.

Laboratory Data

Once the samples are received at SAML, samples are logged and stored as described in Table B2.1 until processed. The COC will be checked for number of samples, proper and exact I.D. number, signatures, dates, and type of analysis specified. If any discrepancy is found, proper corrections will be made. All COC and analytical data will be manually entered into electronic spreadsheets. The electronic spreadsheets will be created in Microsoft Excel software on an IBM-compatible microcomputer with a Windows Operating System. The spreadsheets will be maintained on the computer's hard drive, which is also simultaneously saved in a network folder. Data manually entered in the spreadsheets will be reviewed for accuracy by the Project Co-Leads to ensure that there are no transcription errors. The SAML Lab Director will monitor and evaluate data for all *E. coli*, *Enterococci*, and fecal coliform analyses. Paper and electronic copies of data will be housed in SAML for a period of two years following the conclusion of the project. Any COC's and analysis records related to QA/QC of lab procedures will be housed at SAML. All pertinent electronic data files will be backed up monthly on an external hard drive and stored in separate area away from the computer. Finally, all electronic files will be archived to CD upon completion of the project, and then stored with the final report for 5 years.

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 Co-Leads, TWRI QAO, 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 Section D2.

Data Dissemination

At the conclusion of the project, the Project Co-Leads will provide a copy of the complete project electronic spreadsheet via recordable CD 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

Table C1.1 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	Continuous	TWRI	Monitoring of project status and records to ensure requirements are being fulfilled.	Report to TSSWCB in Quarterly Report.
Internal Monitoring Systems Audit of Program Subparticipants	Dates to be determined by the TWRI	TWRI	Field sampling, handling and measurement; facility review; and data management as they relate to the project	45 days to respond in writing to the TWRI. TWRI will report problems to TSSWCB in Progress Report.
TSSWCB Monitoring Systems Audit	Dates to be determined by TSSWCB	TSSWCB	Field sampling, handling and measurement; facility review; and data management as they relate to the project	45 days to respond in writing to TSSWCB to address corrective actions
Laboratory Inspections	Dates to be determined by TSSWCB	TSSWCB	Analytical and quality control procedures employed at project laboratories	45 days to respond in writing to TSSWCB to address corrective actions

Internal audits of data quality and staff performance to assure that work is being performed according to standards will be conducted by all entities. Audits will be documented and initialed by the pertinent Project Co-Lead. If audits show that the work is not being performed according to standards, immediate corrective action will be implemented and documented.

The TSSWCB QAO (or designee) may conduct an audit of the field or technical systems activities for this project as needed. Each entity will have the responsibility for initiating and implementing response actions associated with findings identified during the on-site audit. Once the response actions have been implemented, the TSSWCB QAO (or designee) may perform a follow-up audit to verify and document that the response actions were implemented effectively. Records of audit findings and corrective actions are maintained by the TSSWCB PM and TWRI QAO. Corrective action documentation will be submitted to the TSSWCB PM 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.

Corrective Action Process for Deficiencies

Deficiencies are any deviation from the QAPP. Deficiencies may invalidate resulting data and may require corrective action. Corrective action may include for samples to be discarded and re-collected. Deficiencies are documented in logbooks, field data sheets, etc. by field or laboratory staff. It is the responsibility of each respective entity's Project Co-Leader or PM, in consultation with 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 PM both verbally and in writing in the project progress reports and by completion of a CAR. All deficiencies identified by each entity will trigger a corrective action plan.

Corrective Action

Corrective Action Reports (CARs) should:

- Identify the problem, nonconformity, or undesirable situation
- Identify immediate remedial actions if possible
- Identify the underlying cause(s) of the problem
- Identify whether the problem is likely to recur, or occur in other areas
- Evaluate the need for Corrective Action
- Use problem-solving techniques to verify causes, determine solution, and develop an action plan
- Identify personnel responsible for action
- Establish timelines and provide a schedule
- Document the corrective action

The status of CARs will be included with quarterly 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.

The Project Co-Lead or PM or each respective entity is responsible for implementing and tracking corrective actions. Records of audit findings and corrective actions are maintained by the Project Co-Lead or PM of each respective entity. Audit reports and corrective action documentation will be submitted to the TSSWCB with the Progress Report.

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 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.

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 of this document. Data review, verification, and validation will be performed using self-assessments and peer and management review as appropriate. The data review tasks to be performed include evaluation of:

- Sample documentation complete; samples labeled
- Field QC samples collected as prescribed in QAPP
- Chain of custody complete
- NELAC Accreditation current
- Holding times not exceeded
- Collection, preparation, and analysis consistent with QAPP
- Bacteriological records complete
- QC samples analyzed at required frequency
- QC results meet performance and program specifications
- Results, calculations, transcriptions checked
- Laboratory bench-level review performed
- All laboratory samples analyzed for all parameters
- Nonconforming activities documented
- Outliers confirmed and documented; reasonableness check performed
- Absence of transcription error confirmed
- Sampling and analytical data gaps checked
- Verified data log submitted
- 10% of data manually reviewed

Potential errors are identified by examination of documentation and by manual or computer-assisted examination of corollary or unreasonable data. If a question arises or an error is identified, the Project Co-Lead responsible for generating the data will work to resolve the issue. Issues which can be corrected are corrected and documented. If an issue cannot be corrected, the responsible Project Co-Lead will consult with the Project Team to establish the appropriate course of action, or the data associated with the issue are rejected and not reported to the TSSWCB. Field and laboratory reviews, verifications, and validations are documented.

After the field and laboratory data are reviewed, another level of review is performed once the data are combined into a data set. This review step is performed by the Project Team. Data review, verification, and validation tasks to be performed on the data set include, but are not limited to, the confirmation of laboratory and field data review, evaluation of field QC results, additional evaluation of anomalies and outliers, analysis of sampling and analytical gaps, and confirmation that all parameters and sampling sites are included in the QAPP.

Another element of the data validation process is consideration of any findings identified during the monitoring systems audit conducted by the TSSWCB. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be

assessed. After the data are reviewed and documented, the Project Team validates that the data meet the data quality objectives of the project and are suitable for reporting to TSSWCB.

If any requirements or specifications of the QAPP are not met, based on any part of the data review, it will be documented and submitted to the TSSWCB with the data. This information is communicated to the TSSWCB by the TWRI in the QA section of the Final Report.

D3 RECONCILIATION WITH USER REQUIREMENTS

Data produced in this project will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be used by Extension to design education programs based on 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 bacterial contamination of streams from grazing lands. No other decisions will be made by the project team based on the data collected. 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. ISCO® SAMPLER MAINTENANCE

General Maintainance Form

Date _____ Time _____

Site Name _____ Observer's Name _____

Grazing Field Heavy Moderate Non-Grazed

Runoff Event Yes No

Sampler Display _____

Level (ft) _____ Zeroed Yes No

Flow _____ cf

FlowMeter Downloaded Yes No

Sampler Reset to Disabled Yes No

Pump Tubing _____ OK Needs Changed

Battery _____ v Solar Panel Clean Obstructed

Dessicants OK Changed Needs Changed

Bubble Rate Fast OK Slow

Cattle In Plots Yes No

Water Full Needs Water

Electric Fence On Off Needs Repair Voltage Reading _____

Fence Upright Yes No

Date Cattle Moved In _____ Time _____ Beginning Grass Height ( )in

Date Cattle Moved Out _____ Ending Grass Height ( )in

Number of Days Present _____ Number of Cows _____

Grass Height (in) _____

Shelter Condition Stable Level Unlevel

Needs Weedeated Yes No

Bubbler Clear Clogged Needs Repair

Stilling Well Upright Needs Repair

V-Wier Clear Obstructed Needs Repair

PVC Pipes Connected Needs Repair

Strainer Clear Silted Needs Repair

Weather Clear Partly Cloudy Rain

Wind Intensity Calm Slight Moderate Severe

Soil Condition Cracked Dry Intermediate Damp Wet/ Soggy

Days Since Last Rain 1 2 3 4 5 6 7 >7

Comments: Fecal Coliform _____ cfu/100ml

E-Coli _____ cfu/100ml

Enterococcus _____ cfu/100ml

APPENDIX E. *Bacteroidales* PCR

Bacteroidales 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 *Bacteroidales* 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.

Bacteroidales 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:
   - Plasmid DNA containing 16S rRNA genes from *Bacteroides* are run as standards using 10-fold dilutions of the plasmid ranging from 2.5×10^7 to 25 copies per PCR.

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).

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.