Clean Water Act §319(h) Nonpoint Source Grant Program

Development of a Watershed Protection Plan for Attoyac Bayou

TSSWCB Project # 09-10
Revision 3

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

prepared by

Texas AgriLife Research
Texas Water Resources Institute

the

Texas A&M University Department of Biological and Agricultural Engineering

and the

Stephen F. Austin State University Arthur Temple College of Forestry and Agriculture

Effective Period: November 2009 to April 2014

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

Lucas Gregory
TWRI Quality Assurance Officer
2260 TAMU
College Station, TX 77843-2260
lfgregory@ag.tamu.edu

This Page Left Blank Intentionally

Section A1 Approval Sheet

Quality Assurance Project Plan (QAPP) for the *Development of a Watershed Protection Plan for Attoyac Bayou* project.

United States Environmental Protection Agency (USEPA), Region VI

Name: Curry Jones
Title: USEPA Chief State/Tribal Programs Section

Signature: _____ Date:_____

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

Signature:_____Date:_____

Texas AgriLife Research – Texas Water Resources Institute (TWRI)

Name: Kevin Wagner
Title: Associate Director

Signature:_____Date:_____

Name: Lucas Gregory
Title: TWRI PM & QAO

Signature:_____Date:_____

Texas AgriLife Research – Dept. of Biological and Agricultural Engineering (BAEN)

Name: R. Karthikeyan
Title: Associate Professor

Signature:_____Date:_____

Texas AgriLife Research – Dept. of Soil and Crop Sciences (SCSC)

Name: Terry Gentry
Title: Assistant Professor and Soil and Aquatic Microbiology Lab (SAML) Director

Signature:_____Date:_____

Stephen F. Austin State University – Arthur Temple College of Forestry and Agriculture (SFASU) & Waters for East Texas Center (WET)

Name: Matthew McBroom
Title: Assistant Professor

Signature:_____Date:_____

Name: J. Leon Young
Title: Director, SFASU Soil, Plant, and Water Analysis Laboratory (SPWAL)

Signature:_____Date:_____

Castilaw Environmental Services, LLC (CES)

Name: Anthony Castilaw
Title: President

Signature:_____Date:_____

Angelina-Neches River Authority (ANRA)

Name: Brian Sims
Title: ANRA PM & QAO

Signature:_____Date:_____

Name: Trey Reeves
Title: Lab Manager (LM)

Signature:_____Date:_____

Section A2: Table of Contents

A1 Approval Sheet 3
A2 Table of Contents 5
List of Acronyms and Abbreviations 7
A3 Distribution List 8
A4 Project/Task Organization 10
A5 Problem Definition/Background 15
A6 Project/Task Description 17
A7 Quality Objectives and Criteria 39
A8 Special Training/Certifications 46
A9 Documentation and Records 47
B1 Sampling Process Design (Experimental Design) 51
B2 Sampling Method Requirements 56
B3 Sample Handling and Custody Requirements 61
B4 Analytical Methods 63
B5 Quality Control Requirements 67
B6 Equipment Testing, Inspection, & Maintenance 77
B7 Instrument Calibration and Frequency 79
B8 Inspection/Acceptance Requirements for Supplies and Consumables 81
B9 Data Acquisition Requirements (Non-direct Measurements) 82
B10 Data Management 85
C1 Assessments and Response Actions 91
C2 Reports to Management 93
D1 Data Review, Verification and Validation 94
D2 Validation Methods 96
D3 Reconciliation with User Requirements 100

References 102

Appendix A Corrective Action Report 105
Appendix B Land Use / Land Cover Field Sampling Form 109
Appendix C Surface Water Quality Monitoring Field Data Sheet 113
Appendix D Chain of Custody Records 117
Appendix E BST Standard Operating Procedures 121
E1: Archival of *Escherichia coli* Isolates 123
E2: ERIC-PCR of *Escherichia coli* 125
E3: RiboPrinting of *Escherichia coli* 130
E4: *Bacteroidales* PCR 147
Appendix F Sample Handling and Shipping for EPA Method 1603 152
Appendix G Data Review Check List and Data Summary Sheet 156
Appendix H RUAA Documentation 162
Appendix I Attoyac Bayou Watershed RUAA Field Survey Sites Distribution 186

List of Tables

Table A6.1 Project Plan Milestones 26
Table A6.2 Attoyac Bayou Sampling Site Locations 29
Table A6.3 Attoyac Bayou Watershed RUAA Field Survey Sites 32
Table A6.4 Big Iron Ore Creek RUAA Field Survey Sites 33
Table A6.5 Naconiche Creek RUAA Field Survey Sites 33
Table A6.6 Terrapin Creek RUAA Field Survey Sites 34
Table A6.7 Waffelow Creek RUAA Field Survey Sites 34
Table A6.8 West Creek RUAA Field Survey Sites 35
Table A7.1 Measurement Performance Specifications 44
Table A7.2 Measurement Performance Specifications for BST Analysis 45
Table A9.1 Project Documents and Records 49
Table B1.1 Waterborne Constituents Evaluated 53
Table B1.2 Samples to be Analyzed using *Bacteroidales* PCR and ERIC-RP 55
Table B2.1 Storage, Preservation and Handling Requirements 58
Table B4.1 Laboratory Analytical Methods 66
Table B6.1 Equipment Inspection and Maintenance Requirements 78
Table B7.1 Instrument Calibration Requirements 80
Table C1.1 Assessments and Response Actions 91
Table D1.1 Ground Control Point Data Review, Validation and Verification Criteria 94
Table D2.1 Data Review Tasks 97

List of Figures

Figure A4.1 Project Organization Chart 14
Figure A6.1 Attoyac Bayou Watershed 20
Figure A6.2 Flow Duration Curve 30
Figure A6.3 Load Duration Curve 31
Figure A6.4 Flow Diagram of Experimental Approach for BST 38
Figure B10.1 Information Dissemination Diagrams 89

List of Acronyms and Abbreviations

ANRA	Angelina-Neches River Authority
AWRL	ambient water reporting limits
BAEN	Department of Biological and Agricultural Engineering
BMP	best management practice
BSLC	Bacteria Source Load Calculator
BST	bacterial source tracking
CAR	corrective action report
CES	Castilaw Environmental Services, LLC.
COC	chain of custody
CRP	Clean Rivers Program
CWA	Clean Water Act
DO	dissolved oxygen
DOQQ	digital ortho quarter quad
DQO	data quality objectives
DTED	digital terrain elevation data
EP-AREC	El Paso AgriLife Research and Extension Center
ERIC-PCR	enterobacterial repetitive intergenic consensus PCR
ERIC-RP	ERIC-PCR / RiboPrinting combination method
FDC	flow duration curve
GIS	geographic information system
GPS	global positioning system
GSD	ground sample distance
LCSD	laboratory control sample duplicate
LDC	load duration curve
LIMS	laboratory information management system
LM	Laboratory Manager
LOQ	limit of quantitation
LULC	landuse/landcover
mTEC	membrane Thermotolerant *E. coli*
MUG	4-methylumbelliferyl-β-D-glucuronide
NAD	North American Datum
NAIP	National Agriculture Imagery Program
NDOP	National Digital Orthophoto Program
NHD	National Hydrography Dataset
NIST	National Institute of Standards and Technology
NLCD	National Land Cover Data set
NRCS	Natural Resource Conservation Service
PDOP	position dilution of precision
PM	Project Manager
QA	quality assurance
QAPP	quality assurance project plan
QAO	Quality Assurance Officer
QC	quality control
QM	quality manual
QPR	quarterly progress report
RC&D	resource conservation & development
RPD	relative percent difference
RUAA	recreational use attainability analysis
SAML	Soil and Aquatic Microbiology Lab
SCSC	Department of Soil and Crop Sciences
SELECT	Spatially Explicit Load Enrichment Calculation Tool
SFASU	Stephen F. Austin State University
SLOC	station location
SM	Standard Methods for Examination of Water and Wastewater, 21st Edition
SOP	standard operating procedure
SPWAL	Soil, Plant and Water Analysis Laboratory
SSURGO	soil survey geographic
SWCD	Soil and Water Conservation District
SWQMIS	surface water quality monitoring information system
TAMU	Texas A&M University, College Station Campus
TCEQ	Texas Commission on Environmental Quality
TCEQ SOP V1	TCEQ's Surface Water Quality Monitoring Procedures, Volume 1
TFS	Texas Forest Service
TMDL	total maximum daily load
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas AgriLife Research, Texas Water Resources Institute
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UTM	Universal Transverse Mercator
WET	Waters of East Texas Center
WPP	watershed protection plan
WWTF	wastewater treatment facility
%R	percent recovery

Section A3: Distribution List

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

United States Environmental Protection Agency, Region VI

6WQ-AT
1445 Ross Avenue, Suite 1200
Dallas, TX 75202-2733

Name: Curry Jones
Title: USEPA Chief State/Tribal Programs Section

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

Texas State Soil and Water Conservation Board

PO Box 658
Temple, TX 76503

Name: Mitch Conine
Title: TSSWCB PM

Name: Pamela Casebolt
Title: TSSWCB QAO

Texas AgriLife Research - Texas Water Resources Institute

2260 TAMU
College Station, TX 77843-2260

Name: Kevin Wagner
Title: Associate Director; Project Lead

Name: Lucas Gregory
Title: TWRI PM & QAO

Texas AgriLife Research - Department of Biological and Agricultural Engineering

306A Scoates
2117 TAMU
College Station, TX 77843-2117

Name: R. Karthikeyan
Title: Associate Professor; Project Co-Leader

Texas AgriLife Research - Department of Soil and Crop Sciences

370 Olsen Blvd.
2474 TAMU
College Station, TX 77843-2474

Name: Terry Gentry
Title: Assistant Professor; Project Co-Lead

Stephen F. Austin State University – Arthur Temple College of Forestry and Agriculture

PO Box 6109, SFA Station,
Nacogdoches, TX 75962-6109

Name: Matthew McBroom
Title: Assistant Professor; Project Co-Lead

Name: J. Leon Young
Title: Director, SFASU SPWAL

Castilaw Environmental Services, LLC.

PO Box 631025
Nacogdoches, TX 75963

Name: Anthony Castilaw
Title: President

Angelina-Neches River Authority

PO Box 387
Lufkin, TX 75901

Name: Brian Sims
Title: ANRA PM and QAO

Name: Trey Reeves
Title: ANRA LM

Section A4: Project/Task Organization

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

USEPA – Provides project oversight and funding at the federal level.

Henry Brewer, USEPA Texas Nonpoint Source Project Officer
Responsible for overall performance and direction of the project at the federal level. Ensures that the project assists in achieving the goals of the Clean Water Act (CWA). Reviews and approves the QAPP, project progress, and deliverables.

TSSWCB – Texas State Soil and Water Conservation Board, Temple, Texas. Provides project overview at the State level.

Mitch Conine, TSSWCB PM
Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified. Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants.

Pamela Casebolt; TSSWCB QAO
Reviews and approves QAPP and any amendments or revisions. Responsible for verifying that the QAPP is followed by project participants. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures. Determines that the project meets the requirements for planning, quality assessment, quality control (QC), and reporting under the TSSWCB Total Maximum Daily Load Program.

TWRI – Texas Water Resources Institute, College Station, Texas. Responsible for general project oversight, coordination administration, reporting and development of data quality objectives (DQOs) and a QAPP.

Kevin Wagner, TWRI Associate Director
The TWRI Project Lead is responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC 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 PM.

Lucas Gregory, TWRI PM & QAO
Responsible for determining that the QAPP meets the requirements for planning, QA and QC. Conducts audits of field and laboratory systems and procedures. Responsible for maintaining the official, approved QAPP, as well as conducting quality assurance audits in conjunction with TSSWCB personnel. Also responsible for supporting the development and ensuring the timely delivery of project deliverables, ensuring cooperation between project partners, providing fiscal oversight and completing project reporting.

BAEN – Department of Biological and Agricultural Engineering, Texas A&M University, College Station, Texas. Responsible for modeling activities associated with the Spatially Explicit Load Enrichment Calibration Tool (SELECT) and Load Duration Curve (LDC) development.

R. Karthikeyan, Associate Professor, BAEN; Project Co-Lead

Responsible for performing LDC analysis and SELECT modeling. This includes ensuring that personnel involved in qualitative data assessment are adequately trained and a thorough knowledge of the QAPP and its requirements specific to the analysis or tasks performed. Responsible for modeling oversight and 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.

SCSC – Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas. Responsible for bacterial source tracking (BST) analysis and inclusion of fecal samples into the Texas Known Source Library.

Terry Gentry, Assistant Professor, SCSC; SAML Director; Project Co-Lead

Responsible for performing BST analysis and related activities. This includes ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and its requirements 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 DQOs in the QAPP. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems.

SFASU – Arthur Temple College of Forestry and Agriculture and the Waters for East Texas Center at Stephen F. Austin State University, Nacogdoches, Texas. Responsible for collecting environmental data, preparing bacteria samples for BST; also oversight of WET Lab activities.

Matthew McBroom, Assistant Professor, SFASU; Project Co-Lead

Responsible for overseeing the installation and operation of environmental monitoring equipment and carrying out scheduled routine monitoring, sample collection, sample preparation and coordinating delivery of collected samples to ANRA. Also responsible for conducting RUAAs in conjunction with CES and summarizing findings. This includes ensuring that field and laboratory personnel involved in collecting and processing environmental samples have adequate training and thorough knowledge of the QAPP and its requirements specific to the task or analysis performed. Responsible for oversight of all field and laboratory operations ensuring that all QA/QC requirements are met, documentation

related to the data collection and analysis are complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified.

J. Leon Young, Director, SPWAL

Monitors all sample analysis within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems. Ensures that adequate training and thorough knowledge of the QAPP and its requirements specific to the analysis performed are fully understood. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the data collection and analysis are 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 DQOs in the QAPP. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems. Ensures that proper shipping procedures are utilized in sending prepared samples to SAML.

CES – Castilaw Environmental Services, LLC. Nacogdoches, Texas. Responsible for developing updated landuse/landcover (LULC) maps for the Attoyac Bayou watershed and developing a GIS inventory for the watershed.

Anthony Castilaw, President, Castilaw Environmental Services, LLC.

Responsible for collaborating in conducting the field work and surveys needed to develop RUAAs for multiple locations within the Attoyac Bayou watershed, providing oversight for the development of a GIS inventory the watershed and updating the current LULC maps for the watershed. Responsible for ensuring that all guidelines and QA/QC requirements set forth in the QAPP are met and followed related to the collection of field data, GIS inventory development and LULC map updates.

ANRA – Angelina-Neches River Authority, Lufkin, Texas. Responsible for conducting water quality analysis, maintaining a water quality database and transmitting project data to TSSWCB in a format such that it is ready for submission to the Texas Commission on Environmental Quality (TCEQ) for inclusion in their Surface Water Quality Monitoring Information System (SWQMIS).

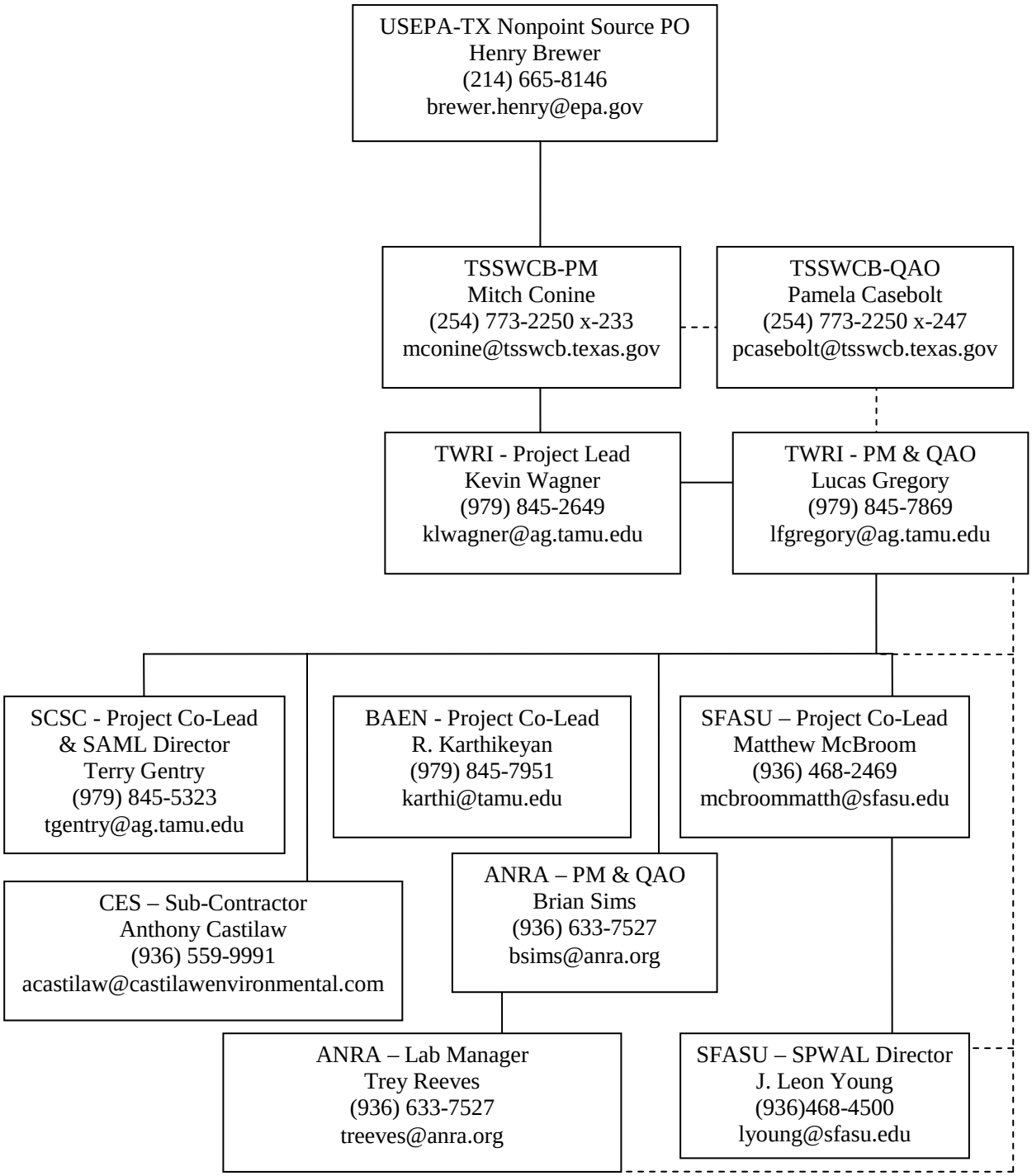
Brian Sims, PM & QAO, Angelina Neches River Authority

Responsible for ensuring that project tasks, deliverables and requirements are met on time and as described. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the data collection and analysis are 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 DQOs in the QAPP. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems.

Trey Reeves, LM, Angelina Neches River Authority

Responsible for coordinating the receipt of water samples from SFASU and performing required analytical analysis on all samples received. Also responsible for assimilating and storing environmental water quality data in a form such that it is prepared for delivery to TCEQ. This includes ensuring that laboratory personnel involved in processing environmental samples have adequate training and thorough knowledge of the QAPP and its requirements specific to the analysis performed.

Figure A4.1. Project Organization Chart



Section A5: Problem Definition/Background

The Neches River Basin in East Texas originates in Van Zandt County southeast of Dallas and flows in a southeastern direction through the Pineywoods of East Texas to the Gulf of Mexico. The river basin has been divided into an upper and lower portion for management and monitoring purposes. The Angelina-Neches River Authority (ANRA) is responsible for the Upper Neches River Basin (4,768,640 ac.) which extends from the headwaters of the Neches River downstream to its confluence with the Angelina River at B.A. Steinhagen Lake in Tyler and Jasper Counties. Within this area, there are 9 classified segments, 13 monitored tributaries and 4 water supply reservoirs. The watershed is largely situated within the Southern Central Plains eco-region and agricultural and silvicultural related industries and operations dominate the landscape and undoubtedly play a significant role in the watershed's hydrology and quality. Urban sprawl coupled with an increasing number of rural residents and land subdivision is also currently impacting the watershed and its hydrological processes.

The Attoyac Bayou, Segment 0612, is one sub-watershed within the Upper Neches River Watershed that is experiencing changes in its hydrologic regime, and subsequent changes in water quality. Watershed dynamics have changed over time and environmental stressors have been exacerbated thru expanded human influences and increasing demand for water resources, increasing pollutant load and the concentration of pollutant loads. These changes have resulted in the elevation of bacteria and nutrient levels relative to Texas Surface Water Quality Standards. The Bayou extends approximately 82 miles from its headwaters in Rusk County and flows through Nacogdoches, San Augustine and Shelby Counties before emptying into Sam Rayburn Reservoir. The watershed contains several named communities including Chireno, Attoyac, Martinsville, Grigsby, Garrison and others; however, these are small rural communities. Chireno and Garrison are the only two with Census Bureau estimated populations for 2007 of 419 and 858 respectively. The remainder of the area is predominantly managed for agricultural (cattle and poultry), silvicultural, recreational and wildlife uses and contains many rural residents and four known permitted wastewater discharges totaling a maximum of 338,000 gallons per day.

The Attoyac Bayou watershed is one of many rural watersheds that are included in the *Texas Water Quality Inventory and 303(d) List* as an impaired water body due to excessive *E. coli* levels. In many cases the assessed data set includes a relatively small number of water quality samples collected over a 5 to 7 year period. Two Clean Rivers Program (CRP) monitoring sites are operated on a quarterly basis by the ANRA at the US 59 (Station 16076) and SH 7 (Station 15253) road crossings and are used to assess water quality in the Bayou. Another water quality monitoring station is located at the SH 21 crossing (Station 10636) and has been operated by ANRA, Texas Commission on Environmental Quality (TCEQ) and the U.S. Geological Survey (USGS); it is currently being operated by TCEQ. A review of the existing water quality data reveals that in many cases the reported *E. coli* levels are elevated above the *E. coli* single sample limit of 394 cfu/100ml and the geometric mean of all samples collected exceed the state standard of 126 cfu/100ml at all three sites.

Previous projects conducted in the area have laid the ground work and produced project outcomes that will be incorporated into this effort. Specifically, the TSSWCB funded (04-06) project entitled *Modeling Nutrient Loads from Poultry Operations in Toledo Bend Reservoir and Sam Rayburn Reservoir Watersheds* utilized the soil and water assessment tool to simulate flow and nutrient loading in the Sam Rayburn Reservoir watershed (includes the Attoyac Bayou). These data will provide critical flow and nutrient loading information that will aid in the development of feasible best management practices (BMPs) to address bacteria and nutrient loadings and develop expected load reductions for each constituent. In addition, the TSSWCB funded (05-04) project entitled *Texas*

Silvicultural Nonpoint Source Pollution Abatement and Prevention Project was carried out by the Texas Forest Service (TFS) in the greater East Texas area to assess the implementation and effectiveness of forestry related BMPs targeted to improve water quality. Under the proposed effort, TFS will collaborate and provide information on BMP effectiveness and strategies to encourage voluntary implementation of these BMPs.

Although these data tend to justify the currently listed impairment, limited flow data has been collected on the Bayou and as a result, it is difficult to calculate an accurate *E. coli* loading rate and the most likely sources of *E. coli* contamination. The needs for a bolstered data set and comprehensive data analysis arise as management options are considered. Without adequate data, uncertainty increases in properly identifying the sources of contamination in the watershed while comprehensive data analysis is needed to hone in on potential sources of watershed pollutants. Collecting two years of additional water quality and streamflow data along with input from local stakeholders will provide much needed information that will enable more accurate watershed pollutant source assessments and the development of a focused and effective watershed protection plan (WPP).

Section A6: Project Goals and Task Description

This project shall serve as a means for establishing and engaging a watershed stakeholder group to assist in the development and future implementation of a WPP for the Attoyac Bayou watershed (Figure A6.1). This project will utilize portions of the "Three-Tier Approach for Bacteria TMDL Development" as recommended in the *Bacteria TMDL Task Force Report* (Jones et al. 2009) submitted to TCEQ and TSSWCB. Tier 1 and Tier 2 recommended tasks will be combined to develop a better understanding of the hydrology, water quality, potential causes and sources for the impairment and will cultivate stakeholder ideas to include in the development of a WPP for the Attoyac Bayou.

Public participation and stakeholder involvement will be handled primarily by CES who will serve as the Watershed Coordinator and will be assisted by ANRA, BAEN, Pineywoods Resource Conservation and Development (RC&D), SCSC, SFASU and TWRI. A diverse group of landowners, public officials, special interest groups and agencies have been identified as potential participants in a stakeholder group and will be asked to provide guidance for the direction of the project and development of the WPP. Input from stakeholders is critical to the success of all watershed planning and implementation efforts. Stakeholder input will be sought throughout this project extensively. Insight provided by the stakeholders will supply much needed information and greatly assist in identifying potential impairment sources and in determining best management strategies for future implementation. Routine stakeholder meetings will be held to provide information about the project objectives, data analysis results, GIS inventory updates and the final results of the project. Project information will be presented through other avenues as well (ANRA CRP meetings, Regional Water Planning meetings, Special Interest Meetings, project website, etc.).

A comprehensive GIS inventory of the watershed will be developed by CES, utilizing their extensive prior knowledge of the watershed and GIS mapping expertise. They will seek input from local stakeholders, public officials, agency personnel and other means necessary to develop a comprehensive GIS inventory of the watershed that illustrates waterbodies, roadways, permitted point-source dischargers, animal feeding operations and other points of concern. Additionally, CES will update current land use/land cover maps for the watershed and will utilize ground-truthing data points collected for the GIS inventory to verify the accuracy of the LULC map. These data will be provided to BAEN for inclusion in the SELECT model analysis.

A targeted water quality monitoring approach will be employed through this project. This effort will be led by SFASU and coordinated with ANRA CRP personnel to ensure that as much continuity as possible is maintained between sampling efforts. Bi-weekly (twice per month) sampling will be conducted at 10 sampling sites listed in Table A6.2. This increased spatial and temporal sampling will allow for more accurate and realistic comparisons between previously collected data and data collected during the course of this project. In-situ water quality monitoring collected using a YSI multi-probe will include pH, conductivity, dissolved oxygen (DO), and Temperature (^{o}C). Flow measurements will also be taken at each monitoring site and will be critical for the development of LDCs. Water samples will be collected by SFASU and delivered to ANRA to be analyzed for *E. coli* (IDEXX method), ammonia, nitrate, total phosphorus, dissolved orthophosphorus and total suspended solids. Additional water samples collected by SFASU will be taken to the SFASU SPWAL at SFASU and prepared and stored utilizing the USEPA 1603 method for *E. coli* analysis and membrane filtration for *Bacteroidales* PCR; samples will later be transferred to the SAML for BST analysis. These routine data will be supplemented with stormflow samples automatically collected using ISCO automated sampling devices calibrated to collect samples based on water level changes resulting from storm events. Stormflow samples will be collected from a minimum of 10 storm events at 2 locations.

SFASU will transfer all collected water samples to the ANRA labs within their prescribed holding times. The National Environmental Laboratory Accreditation Conference (NELAC) approved ANRA Lab will be responsible for conducting water quality analyses. Data will be stored in a master database maintained by ANRA; field data collected by SFASU will also be transmitted to ANRA for inclusion in their database. ANRA will manage and prepare data consistent with the TCEQ Data Management Reference Guide (DMRG) for submittal to TSSWCB and transmittal to TCEQ for inclusion into SWQMIS.

Analyzing historic data and data collected during the 2-year monitoring period will be conducted through this project. BAEN will develop LDCs for *E. coli* and ammonia using available historic data and estimated flow readings for each of the three previously monitored sites and will develop updated LDCs for all sampling sites after water quality sampling and flow monitoring has been completed. The developed LDCs will be consistent with EPA's *Approach for Using Load Duration Curves in the Development of TMDLs* (EPA 2007a), EPA's *Options for Expressing Daily Loads in TMDLs* (EPA 2007b) and EPA's *Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality* (EPA 2008). This analysis will provide a goal for needed *E. coli* and ammonia load reductions and aid in identifying potential sources of *E. coli* and ammonia based on flow conditions.

BAEN will also be responsible for evaluating *E. coli* contamination sources in Attoyac Bayou watershed using SELECT. Information collected in the development of the GIS inventory, LU/LC update, SWQM and LDC development will be incorporated into SELECT to determine *E. coli* loads for specific areas of the watershed. The SELECT approach will also provide an appropriate ranking of each pollutant source based on its potential to contribute to the overall *E. coli* loading in the watershed.

To assess and identify different sources contributing to bacteria loadings, SAML will conduct Bacterial Source Tracking (BST) in the study area. SAML will conduct library-independent BST utilizing the *Bacteroidales* PCR genetic test for human, ruminant, horse, and swine markers. Additionally, SAML will conduct limited library-dependent BST and analyze *E. coli* isolates utilizing the ERIC-PCR and RiboPrinting combination method. This will serve to confirm that the sources of *E. coli* and *Bacteroidales* are comparable and assess the spatial and temporal adequacy of the Texas Known Source Library. The Texas Known Source Library may need to be supplemented with known fecal samples from the study area. The SFASU SPWAL will provide SAML a subset of water samples. Additionally, the WET Lab will prepare known fecal samples for inclusion in the Texas Known Source Library. Results from the source survey will be used by SAML to make appropriate adjustments to the BST sampling design and to assess the adequacy of the Texas Known Source Library. SAML will work with BAEN to integrate BST results into the model, to the extent possible, and address and reconcile discrepancies between BST and modeling results.

CES and SFASU will conduct a Comprehensive Recreational Use Attainability Analysis (RUAA) to assess the physical, chemical, biological, and economic factors affecting attainment of recreation use in the Attoyac Bayou (Segment 0612), Terrapin Creek (Segment 0612A), Waffelow Creek (Segment 0612B), Naconiche Creek, Big Iron Ore Creek and West Creek. Methods used shall be consistent with the TCEQ *Recreational Use-Attainability Analyses – Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey* (TCEQ 2009). CES and SFASU shall conduct a thorough historical information review of the recreational uses of the waterbody that occurred on and/or after November 28, 1975. CES and SFASU will conduct field surveys at selected sites during the period people would most likely be using the waterbody for contact recreation (Spring and Summer). Field surveys shall

ascertain the suitability of the streams for contact recreation use and shall document the hydrological characteristics of the stream, such as width and depth of channel, flow/discharge, and bank access. A digital photographic record of each selected site shall be collected during the field surveys. In order to obtain information on existing and historical uses and stream characteristics, interviews of: 1) users present during the field surveys, 2) streamside landowners along the field survey transects, 3) local residents, and 4) commercial providers of outdoor recreation goods and services; shall be conducted.

The culminating deliverable for the project will be the development of a stakeholder driven WPP for the Attoyac Bayou that satisfies EPA's nine key elements for developing WPPs. This plan will include information and results from all project tasks and will be based on decisions made by the stakeholder group as a means to manage their watershed resources in the best manner that they see fit while achieving water quality goals and standards.

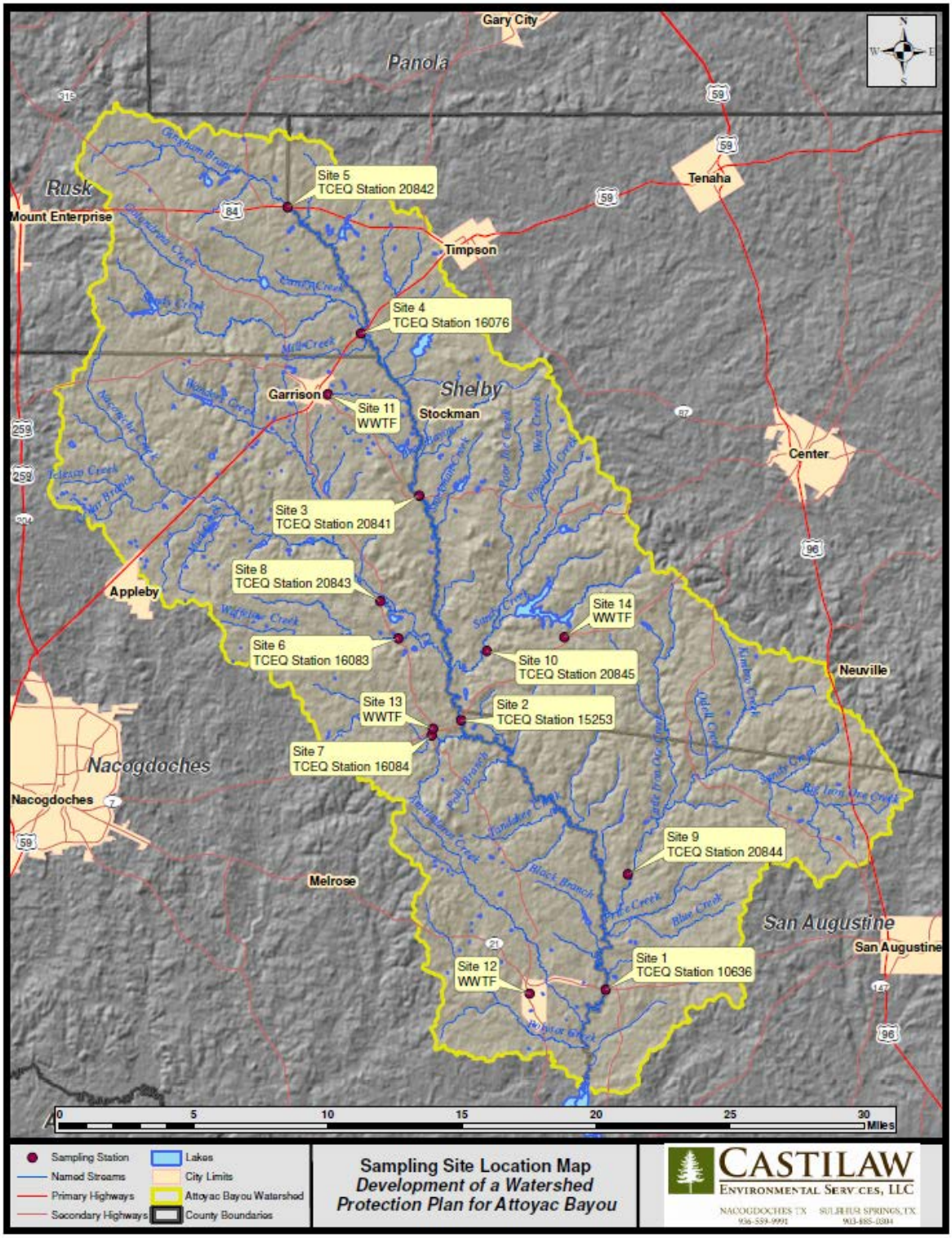


Figure A6.1. Attoyac Bayou watershed

Task 4: Landuse/Landcover Update

Objective: To develop a comprehensive GIS inventory of the watershed including a survey of potential pollutant contributing areas and a Land Use/Land Cover update.

Subtask 4.2: CES will work to update existing Land Use/Land Cover for the watershed to a level that is representative of current watershed conditions. (Start Date: Month 6; Completion Date: Month 15)

Deliverables:

- Updated LU/LC maps for the Attoyac Bayou watershed and delivered to BAEN for use in SELECT modeling
- Technical report that provides details on the components of the LU/LC update

Task 5: Surface Water Quality Monitoring

Objective: To collect additional surface water quality data to characterize *E. coli* and ammonia loadings across varying flow regimes and temporal periods.

Subtask 5.2: SFASU will conduct routine, bi-weekly (twice monthly), ambient water quality monitoring at 10 locations throughout the Attoyac Bayou watershed (see Table 6.2 in the Project Narrative) over the course of 2 years. Sampling will include routine field parameters (Temp, pH, DO, conductivity, flow) and collection of water samples of the volume required by the QAPP. Water samples will be delivered to ANRA within the appropriate holding time for bacteriological and nutrient analysis (these analysis will include ammonia N, nitrate-nitrite N, dissolved Ortho-P, Total P, Total Suspended Solids, and *E. coli* enumeration utilizing the IDEXX method). 52 sampling events are scheduled for a total of 520 samples. Sampling efforts will be coordinated with ANRA and TCEQ. (Start Date: Month 6; Completion Date: Month 30)

Additionally, a subset of water samples (250) will be collected for BST analysis. All 250 samples will be prepared for *Bacteroidales* analysis (Subtask 8.1) and a 100 sample subset of the 250 total samples collected will be prepared for *E. coli* analysis (Subtask 8.2). SFASU will deliver these samples to the SFASU SPWAL for preparation and storage utilizing the USEPA 1603 method for *E. coli* and membrane filtration for *Bacteroidales* PCR. Samples will be periodically transferred to the SAML at TAMU for BST analysis (Task 8). (Start Date: Month 6; Completion Date: Month 30)

Subtask 5.3: SFASU will utilize automated sampling devices to collect stormflow samples at two locations (Attoyac Bayou @ SH 7 and Big Iron Ore Creek @ FM 354). These samples will be picked up by SFASU and delivered to ANRA for analysis. It is anticipated that a minimum of 10 stormflow events will be sampled from each selected site yielding at least 20 total stormflow samples. These samples will be analyzed for the same parameters as listed in Subtask 5.2. (Start Date: Month 6; Completion Date: Month 30)

Subtask 5.4: SFASU will collect water quality samples quarterly for five quarters from the four identified point source dischargers in the watershed. Sampling will include routine field parameters (Temp, pH, DO, conductivity) nutrient parameters and bacteria parameters. Water samples will be delivered to ANRA within the appropriate holding time for bacteriological and

nutrient analysis. 20 samples have been budgeted for. (Start Date: Month 6; Completion Date: Month 21)

Subtask 5.5: ANRA will maintain a master database for housing all environmental water quality data collected through the project. SFASU will maintain a database of field parameter data collected under the project and transmit this data to ANRA for inclusion into the master database. Data collected and analyzed will be included in ANRA's CRP database and submitted to TSSWCB for transmittal to TCEQ for inclusion in SWQMIS. Data will be formatted consistent with TCEQ DRMG. A Station Location (SLOC) Request for any new monitoring stations will be submitted to TCEQ by SFASU (Subtask 5.1). (Start Date: Month 6; Completion Date: Month 36)

Deliverables:

- Completed SLOC request
- Electronic monitoring data files and data summary
- Technical Report summarizing water quality data findings.

Task 6: LDC and SELECT Data Analysis

Objective: To analyze stormflow, *E. coli*, and ammonia data using LDCs and SELECT to determine needed load reductions for ammonia and *E. coli* levels to achieve environmental goals established by stakeholders in the WPP and to estimate potential loadings from identified pollutant sources.

Subtask 6.1: BAEN, with cooperation from other project partners, will develop LDCs on currently available ammonia and bacteria data for each monitoring site on the Attoyac Bayou. LDCs developed will be consistent with *An Approach for Using Load Duration Curves in the Development of TMDLs* (EPA 2007a), *Options for Expressing Daily Loads in TMDLs* (EPA 2007b), and *Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality* (EPA 2008). (Start Date: Month 6; Completion Date: Month 12)

Subtask 6.2: BAEN, with cooperation from other project partners, will update LDCs developed using historic water quality data with water quality data collected under Task 5. LDCs will be used to estimate needed load reduction for ammonia and bacteria at each monitoring site in the waterbody. (Start Date: Month 24; Completion Date: Month 30)

Subtask 6.3: BAEN, with cooperation from other project partners, will conduct watershed modeling using the SELECT approach for the Attoyac Bayou. Information collected in Tasks 4, 5, 7 and 8 will be incorporated with information from LDC analyses to estimate pollutant loadings from various sources within the watershed and identify potentially critical loading areas. (Start Date: Month 24; Completion Date: Month 30)

Deliverables

- Technical report detailing the results of LDC and SELECT analyses

Task 7: Recreational Use Attainability Analysis

Objective: To conduct a Comprehensive RUAA to assess the physical, chemical, biological, and economic factors affecting attainment of recreation use in the Attoyac Bayou (Segment 0612), Terrapin Creek (Segment 0612A) and Waffelow Creek (Segment 0612B).

Subtask 7.2: CES and SFASU shall conduct a thorough historical information review of the recreational uses of the waterbody that occurred on and/or after November 28, 1975. Historical resources that should be examined include, but are not limited to, photographic evidence, local newspapers, museum collections, published reports, historical society records, and long-term landowners/residents. Texas Parks & Wildlife Department and commercial providers of outdoor recreation goods and services should be consulted for historical information.

Subtask 7.3: CES and SFASU will conduct 2 field surveys at each selected RUAA site. Surveys shall be conducted during a normal (average) warm season (air temperature $\geq$ 70°F) during baseflow conditions. Baseflow conditions are sustained or typical dry, warm-weather flows between rainfall events, excluding unusual antecedent conditions of drought or wet weather. The surveys should be performed during the period people would most likely be using the waterbody for contact recreation, typically March to October (e.g., spring break, summer, holidays or weekends).

To ascertain the suitability of the streams for contact recreation use, field surveys shall document hydrological characteristics of the stream, such as width and depth of channel, flow/discharge, air/stream temperature, bank access, and stream substrate. Information to be collected shall satisfy those questions found on the Field Data Sheet found in TCEQ's *Recreational Use-Attainability Analyses (UAAs) – Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey* (TCEQ 2009).

CES and SFASU shall document and describe antecedent (prior to fieldwork) rainfall conditions (approximately the previous 30 days) at each selected site.

Subtask 7.4: CES and SFASU shall collect a digital photographic record of each selected site during the field surveys. Photographs shall include upstream, left and right bank, and downstream views. Any evidence of observed uses or indications of human use shall be photographed. Photographs should clearly depict the entire channel and each transect measured.

Subtask 7.5: In order to obtain information on existing and historical uses and stream characteristics, CES and SFASU shall conduct interviews of 1) users present during the field surveys, 2) streamside landowners along the field survey transects, 3) local residents, and 4) commercial providers of outdoor recreation goods and services. Surveys shall include those questions found on the Interview Form found in TCEQ's *Recreational Use-Attainability Analyses (UAAs) – Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey* (TCEQ 2009).

Deliverables:

- Station Location Request Forms (as needed) in electronic format (if needed; see Task 5)
- Field Data Sheets and Data Summary in electronic format
- Digital photographic record

- Interview Forms and Data Summary in electronic format
- Technical Report summarizing historical information review, field surveys, and user interview

Task 8: Bacterial Source Tracking

Objective: To conduct Bacterial Source Tracking to assess and identify different sources contributing to bacteria loadings.

Subtask 8.1: SAML will conduct library-independent BST on 250 water samples utilizing the *Bacteroidales* PCR genetic test for human, ruminant, horse, and swine markers. The number of samples collected from each location may be adjusted depending on the size of each watershed in the study area and the complexity of sources as identified in the source survey (Task 4). Budgeted number of samples is 20 from each of Terrapin, Waffelow, Naconiche, Big Iron Ore and West Creeks for a total of 100 samples from the tributaries; 125 samples will be collected and analyzed from the Attoyac Bayou (25 from each sampling site); 21 stormflow samples as collected by automated equipment; 4 samples collected from wastewater treatment facilities (WWTFs); in total, 250 samples will be analyzed utilizing *Bacteroidales* PCR. Specific genetic markers for various animal sources are continually being developed by the scientific community and as new markers are identified, they should be included in this analysis as the budget allows. Water samples for this subtask shall be a subset of those collected by SFASU under Task 5. (Start Date: Month 6; Completion Date: Month 30)

Subtask 8.2: SAML will conduct limited library-dependent BST and analyze *E. coli* isolates from 100 water samples (1 isolate per water sample) from across the study area utilizing the ERIC-PCR and RiboPrinting combination method. Isolates will be obtained from water samples collected at: each sampling site (8 samples from each, total of 80 samples), automated stormflow samples (8 samples from each, total of 16 samples) and 1 from each of the 4 WWTFs; yielding a total of 100 samples. This will serve to 1) confirm that the sources of *E. coli* and *Bacteroidales* are comparable and 2) assess the spatial and temporal adequacy of the Texas Known Source Library. (Start Date: Month 6; Completion Date: Month 30)

Subtask 8.3: SAML will add up to 30 known source fecal samples (1-2 isolates per fecal sample) to the Texas Known Source Library. Fecal samples will be added to the BST library utilizing the ERIC-PCR and RiboPrinting combination method. Samples for this subtask shall be collected by CES or SFASU under Task 5. (Start Date: Month 6; Completion Date: Month 30)

Subtask 8.5: BAEN will conduct watershed modeling for the study area (Task 6). SAML will work with BAEN to 1) integrate BST results into the model, to the extent possible, and 2) address and reconcile discrepancies between BST and modeling results. (Start Date: Month 7; Completion Date: Month 21)

Subtask 8.6: CES and SFASU, as appropriate, will collect known source fecal samples from fresh road kill (less than 48 hrs old), known live sources, and other opportunistic sample sources (game taken by hunting or donated by stakeholders) in or very near the watershed. Samples will be delivered to the WET Lab at SFA for processing before being sent to the SAML at Texas A&M University in College Station. (Start Date: Month 6; Completion Date: Month 30)

Deliverables

- Technical report detailing the results of Bacterial Source Tracking
- Known source fecal isolates added to the Texas BST Library

The purpose of this QAPP is to clearly delineate the QA policy, management structure, and procedures, which will be used to implement the QA requirements necessary to conduct a watershed source survey and update landuse and land cover maps under Task 4; collect and monitor water quality throughout the Attoyac Bayou watershed under Task 5; to analyze watershed and water quality data using Load Duration Curves and spatially explicit modeling under Task 6; and to analyze water and fecal samples collected throughout the watershed utilizing BST under Task 8.

Table A6.1. Project Plan Milestones

Task	Project Milestones	Agency	Start	End
4.2	CES will work to update existing Land Use/Land Cover for the watershed to a level that is representative of current watershed conditions.	CES	Month 6	Month 15
5.2	SFASU will conduct routine, bi-weekly, ambient water quality monitoring at 10 locations; parameters include temperature, pH, DO, conductivity and flow. Samples collected from 52 sampling trips (potential for 520 samples) will be delivered to ANRA for *E. coli* enumeration and nutrient analysis. Additionally, a 250 sample subset of water samples will be prepared for BST analysis by the SFASU WET Lab.	SFASU, ANRA	Month 6	Month 35
5.3	SFASU will collect stormflow samples at 2 locations utilizing automated sampling devices; samples will be delivered to ANRA for analyses as described in Subtask 5.2.	SFASU, ANRA	Month 6	Month 35
5.4	SFASU will collect water samples quarterly for five quarters at four identified point source discharges. Filed parameters will be collected and samples will be sent to ANRA for analysis as in Subtask 5.2.	SFASU, ANRA	Month 6	Month 21
5.5	ANRA will maintain master database of all water quality data. SFASU will maintain database of field parameters and transmit data to ANRA. Data will be submitted to TCEQ for inclusion in SWQMIS.	SFASU, ANRA, TSSWCB	Month 6	Month 54
6.1	BAEN will develop LDCs using currently available (historic) ammonia and bacteria data.	BAEN	Month 6	Month 12
6.2	BAEN will update LDCs developed using historic data to include data collected under Task 5. Needed load reductions will be developed using these updated LDCs.	BAEN	Month 24	Month 36
6.3	BAEN will conduct watershed modeling using the SELECT approach. Watershed information collected in Tasks 4 & 7 plus water quality data collected in Tasks 5 & 8 will be used to estimate watershed pollutant loadings and identify critical loading areas.	BAEN	Month 24	Month 54
7.2	CES and SFASU will conduct historical review of recreational uses since Nov. 28, 1975 including photos, news, reports, historical records and oral accounts.	CES, SFASU	Month 16	Month 40
7.3	CES and SFASU will conduct 2 field surveys at selected RUAA sites during normal warm-season, baseflow conditions during the March to October time period. Surveys will document hydrological features and the ability of the public to access the sites.	CES, SFASU	Month 16	Month 36
7.4	CES and SFASU will photo-document sampling sites focusing on human related evidence.	CES, SFASU	Month 16	Month 36
7.5	CES and SFASU will conduct interviews to document current and historical uses of the waters; persons encountered during surveys, landowners, recreational users and others as appropriate will be interviewed.	CES, SFASU	Month 16	Month 40
8.1	SAML will conduct library-independent BST on 250 water samples utilizing *Bacteroidales* PCR genetic test for human, ruminant, horse and swine markers.	SAML	Month 6	Month 36
8.2	SAML will conduct limited library-dependent BST and analyze *E. coli* isolates from 100 water samples utilizing the ERIC-RP combination method.	SAML	Month 6	Month 36
8.3	SAML will add up to 30 known source fecal samples to the Texas Known Source Library utilizing the ERIC-RP combination method.	SAML	Month 6	Month 36
8.5	BAEN will utilize BST results generated by SAML in watershed modeling and work to reconcile any descrepancies between modeling and BST results.	BAEN, SAML	Month 7	Month 54
8.6	CES and SFASU will collect known source fecal samples from fresh roadkill, live sources and other opportunistic samples in or very near the watershed. Samples will be delivered to the SFASU WET Lab for processing prior to shipment to SAML.	CES, SFASU, SAML	Month 6	Month 36

Land Use/Land Cover Update

The project will classify current land use for the Attoyac Bayou watershed through a combination of satellite based image classification schemes and where needed, "heads-up digitizing" of aerial photos. The land use classification scheme to be used in this delineation will include:

- Developed Open Space - Includes areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20% of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.
- Developed Low Intensity - Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20-49% of total cover. These areas most commonly include single-family housing units.
- Developed Medium Intensity - Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50-79% of the total cover. These areas most commonly include single-family housing units.
- Developed High Intensity- Includes highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80-100% of the total cover.
- Open Water - All areas of open water, generally with less than 25% cover of vegetation or soil.
- Barren Land - (Rock/Sand/Clay) - Barren areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material. Generally, vegetation accounts for less than 15% of total cover and includes transitional areas.
- Forested Land – Areas dominated by trees generally greater than 5 meters tall, and greater than 50% of total vegetation cover.
- Near Riparian Forested Land – Areas dominated by trees generally greater than 5 meters tall, and greater than 50% of total vegetation cover. These areas are found following in near proximity (within 60 ft) to streams, creeks and/or rivers.
- Mixed Forest - Areas dominated by trees generally greater than 5 meters tall, and greater than 20% but less than 50% of total vegetation cover.
- Rangeland – Areas of unmanaged shrubs, grasses, or shrub-grass mixtures
- Managed Pasture - Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20% of total vegetation.
- Cultivated Crops - Areas used for the production of annual crops, such as corn, soybeans, vegetables, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20% of total vegetation. This class also includes all land being actively tilled.
- Pine Plantation – Areas of land dominated by pine trees that have been planted to artificially reforest an area for the purpose of timber production; trees are generally planted in an evenly spaced, systematic manner that is easily distinguishable from native tree stands.

Surface Water Quality Monitoring

SFASU will be responsible for the collection and transport of all water quality data and samples to the respective lab (ANRA Lab or SFASU SPWAL) within appropriate sample holding times and in accordance with this QAPP. Sampling will be conducted routinely at the sampling sites designated in Table A6.2.

For BST analysis purposes, the SFASU SPWAL will receive water and fecal samples and pre-process them for *E. coli* isolation. The lab will also pre-process water samples for *Bacteroidales* PCR analysis. *E. coli* will be isolated from the samples using standard microbiological methods as previously used in TSSWCB and TCEQ BST projects. *E. coli* will be isolated from water samples using USEPA Method 1603 and modified membrane Thermotolerant *E. coli* (mTEC) medium. Fecal specimens or domestic sewage samples will be streaked (resuspended in buffer if necessary) onto modified mTEC medium. The use of modified mTEC medium for isolation of *E. coli* from both water and source samples will help avoid selection of different types of *E. coli* due to different media. Inoculated plates will be incubated at 35±0.5^{o}C for 2 hours to resuscitate stressed bacteria, then incubated at 44.5±0.2^{o}C for approximately 20 to 24 hours. 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 the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with 4-methylumbelliferyl-β-D-glucuronide (MUG) observed by ultraviolet light fluorescence. After pre-processing, the plates will be shipped to SAML which will transfer *E. coli* colonies from the modified mTEC medium onto nutrient agar with MUG to confirm glucuronidase activity and culture purity. Additionally, SFASU SPWAL will pre-process water samples for *Bacteroidales* PCR by passing ~100 ml of each water sample through a 0.2 µm filter to collect biomass and then freezing each filter until shipment to SAML for analysis. The SFASU SPWAL will periodically ship or arrange to deliver bacterial cultures and/or *Bacteroidales* filters following shipping procedures outlined in Appendices E-4 and F to SAML for BST analyses.

Table A6.2. Attoyac Bayou Sampling Site Locations

Site #	TCEQ Station #	Sample Type	Sampling Site Name	GPS Coordinates Lat: 31 ° N	Long: 94 ° W
			Stream Sampling Sites		
1	10636	Routine	Attoyac Bayou @ SH 21	30'15.05"	18'13.99"
2	15253	Routine/ Storm	Attoyac Bayou @ SH 7	*38'54.00"*	23'50.00"
3	20841	Routine	Attoyac Bayou @ FM 138	46'6.61"	25'34.50"
4	16076	Routine	Attoyac Bayou @ US 59	51'24.14"	27'49.89"
5	20842	Routine	Attoyac Bayou @ US 84	55'26.97"	30'41.07"
6	16083	Routine	Waffelow Creek @ FM 95	41'29.99"	26'16.00"
7	16084	Routine	Terrapin Creek @ FM 95	38'20.01"	24'53.08"
8	20843	Routine	Naconiche Creek @ FM 95	42'43.80"	26'57.86"
9	20844	Routine/ Storm	Big Iron Ore Creek @ FM 354	*33'57.43"*	17'22.05"
10	20845	Routine	West Creek @ FM 2913	41'13.10"	23'0.09"
			Wastewater Treatment Facility Sampling Sites		
11	N/A	Quarterly	City of Garrison WWTF	49'21.86"	29'2.82"
12	N/A	Quarterly	Chireno ISD WWTF	30'3.13"	21'6.30"
13	N/A	Quarterly	Martinsville ISD WWTF	38'32.29"	24'52.99"
14	N/A	Quarterly	City of Center WWTF	41'38.80"	19'56.66"

LDC and SELECT Data Analysis

Spatially Explicit Load Enrichment Calculation Tool (SELECT)

The Center for Total Maximum Daily Load (TMDL) and Watershed Studies at Virginia Tech has been involved in TMDL development for bacteria impairments. The Center personnel developed a systematic process for source characterization that includes the following steps:

- inventorying bacterial sources (including livestock, wildlife, humans, and pets);
- distributing estimated loads to the land as a function of land use and source type; and
- generating bacterial load input parameters for watershed-scale simulation models.

This process provides a consistent approach that is necessary to develop comprehensive bacteria TMDLs. The Center personnel developed a software tool, the Bacteria Source Load Calculator (BSLC), to assist with the bacterial source characterization process and to automate the creation of input files for water quality modeling (Zeckoski, et al., 2005). But BSLC does not spatially reference the sources. A spatially-explicit tool, SELECT has been developed by the SSL and BAEN Department at Texas A&M University to calculate contaminant-loads resulting from various sources within a watershed. SELECT spatially references the sources to the sub-watershed level using an ArcGIS 9 platform. Sub-watersheds are delineated using the USEPA BASINS program. SELECT will calculate and allocate potential pathogen loading to a stream from various sources within a watershed. In order to allocate the *E. coli* load throughout the Attoyac Bayou watershed, estimations of the source contributions will be made. This in turn allows the sources and locations to be ranked according to their potential contribution for each sub-watershed. The populations of agricultural animals, wildlife, and domestic pets will be calculated and distributed throughout each watershed according to appropriate land use. Septic system contribution will also be

estimated based on criteria including distance to a stream, soil type, and failure rate. Specific data on septic systems is not available for this watershed; therefore, spatially referenced 911 address locations and 2010 Census block data are used as a surrogate. The 911 data provides general locations while the 2010 Census block data provides an appropriate number of systems. Once the watershed profile is developed for each potential source, the information can be aggregated to the sub-watershed level to identify the top contributing areas in the watershed.

Load duration Curve (LDC)

This is a simple and an effective first-step methodology to obtain data-based TMDLs (Cleland, 2003; Stiles, 2001). A duration curve is a graph that illustrates the percentage of time during which a given parameter's value is equaled or exceeded. For example, a flow duration curve (FDC) (Figure A6.2) uses the hydrograph of the observed stream flows to calculate and depict the percentage of time the flows are equaled or exceeded.

A LDC (Figure A6.3), which is related to the FDC, shows the corresponding relationship between the contaminant loadings and stream flow conditions at the monitoring site. In this manner, it assists in determining patterns in pollution loading (point sources, nonpoint sources, erosion, etc.) depending on the streamflow conditions. Based on the observed patterns, specific restoration plans can be implemented that target a particular kind of pollutant source. For example, if the pollutant loads exceed the allowable loads (see Figure A6.4) for low stream flow regimes, then the point sources such as waste water treatment plants and direct deposition sources (wildlife, livestock) should be targeted for the restoration plans. Another main advantage of the LDC method is that it can also be used to evaluate the current impairment as some percent of samples which exceed the standard, and therefore it allows for the rapid development of TMDLs (Stiles, 2001).

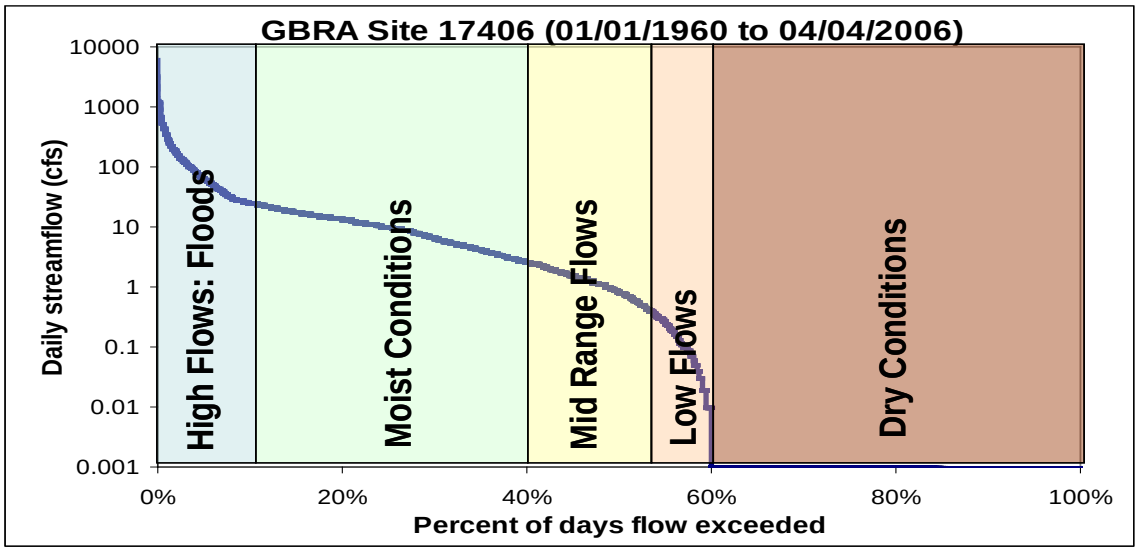


Figure A6.2: Flow Duration Curve (FDC) for streamflow conditions at GBRA monitoring station 17406 on Plum Creek, near Uhland, TX. The flow data at 17406 was obtained from the nearest USGS gage station 8172400, after adjusting for subwatershed aerial contribution during runoff events.

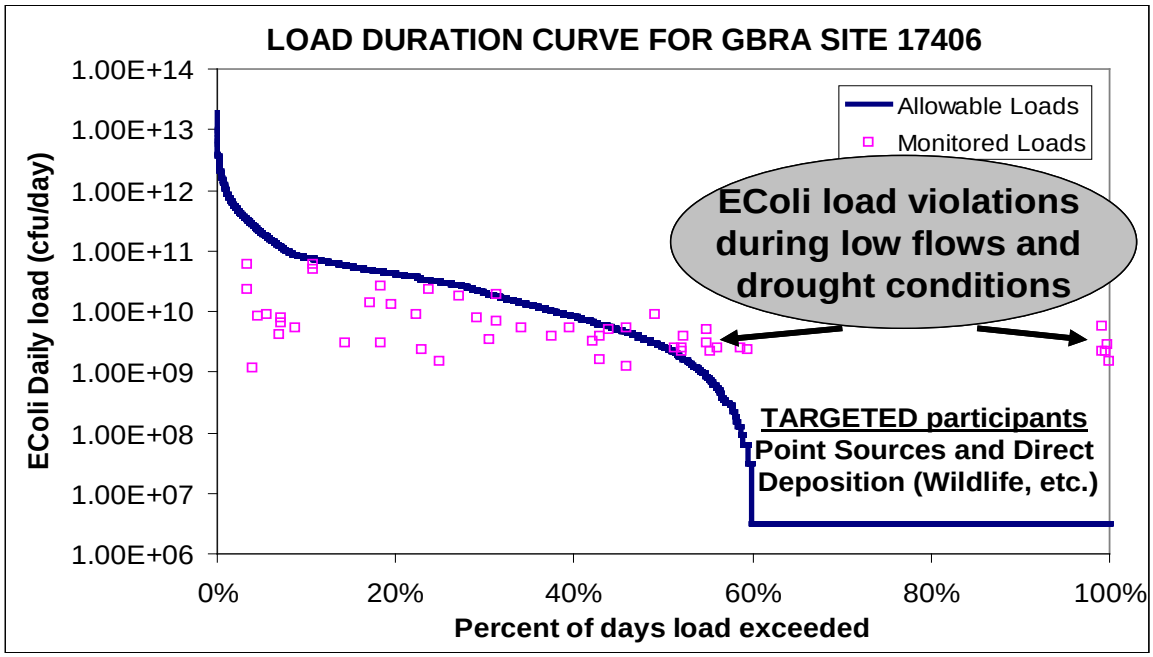


Figure A6.3: Load Duration Curve for *E. coli* at GBRA monitoring station 17406 on Plum Creek, near Uhland, TX. The flow data at 17406 was obtained from the nearest USGS gage station 8172400, after adjusting for subwatershed aerial contribution during runoff events.

RUAA

CES and SFASU will collect information to be used to evaluate factors affecting attainment of recreational uses in the waterbodies in the study area. Methods used will be consistent with the TCEQ *Recreational Use-Attainability Analyses (UAAs) – Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey* (TCEQ 2009).

CES and SFASU shall conduct a thorough historical information review of the recreational uses of the waterbody back to November 28, 1975.

CES and SFASU will conduct 2 field surveys at selected sites defined in Appendix I during the period people would most likely be using the waterbody for contact recreation. Field surveys will ascertain the suitability of the streams for contact recreation use and will document the hydrological characteristics of the stream, such as width and depth of channel and substantial pools, flow/discharge, and bank access. CES and SFASU will collect a digital photographic record of each selected site during the field surveys. In order to obtain information on existing and historical uses and stream characteristics, CES and SFASU will conduct interviews of 1) users present during the field surveys, 2) streamside landowners along the field survey transects, 3) local residents, and 4) commercial providers of outdoor recreation goods and services.

In the interest of generating complete descriptions of all project waterbodies, it is the intent of TSSWCB to fully complete the comprehensive RUAA assessment (inclusive of field surveys

and interviews) on waterbodies where obvious primary contact recreation occurs. This protocol deviates from the guidance in the TCEQ *Recreational Use-Attainability Analyses (UAAs) – Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey* (TCEQ 2009) which suggests terminating the survey when such conditions are encountered. The reason that this deviation will be made is to provide additional descriptive information about waterbodies within the watershed and their use characteristics, both observed and reported by interviewees. This information will be used to better describe physical waterbody conditions, waterbody uses observed and local insight on watershed uses and hindrances to use watershed wide in the Attoyac Bayou WPP.

Table A6.3. Attoyac Bayou RUAA Field Survey Sites

Station Name & Map Legend	*Site ID**	*Site Description*	*Lat: 31 ° N*	*Long: 94 ° W*	*Distance to Previous Site (mi)*
		upper extent of waterbody NHD RC 12020005006317			
0612 AU_03		***AU_03 upper boundary @ FM 95***			-
1 - Attoyac @ FM 95	Attoyac 1	Attoyac Bayou @ FM 95 in Rusk Co. 3.3 mi N of FM 95 - US 84 Intersection	57'6.84"	37'50.88"	*1.1*
2 - Attoyac @ CR 3122	Attoyac 2	Attoyac Bayou @ Rusk CR 3122, 1.6 mi N of CR 3122 - FM 95 Intersection	57'28.44"	36'20.88"	*2.1*
3 - Attoyac @ CR 3181	Attoyac 3	Attoyac Bayou @ Rusk CR 3181, 2.8 mi WSW of CR 3181 - FM 1971 Intersection	57'10.44"	34'54.12"	*2.2*
4 - Attoyac @ FM 1971	Attoyac 4	Attoyac Bayou @ FM 1971, 1.35 mi N of FM 1971 - US 84 Intersection in Caledonia	56'29.07"	32'13.21"	*4.4*
5 - Attoyac @ US 84	20842	Attoyac Bayou @ US 84, 0.9 mi E of US 84 - FM 1971 Intersection in Caledonia	55'26.97"	30'41.07"	*2.8*
6 - Attoyac @ CR 4760	Attoyac 6	Attoyac Bayou @ Rusk CR 4760, 1.3 mi past end of FM 1970 and 4.2 mi W of US 59 - US 84 Intersection	53'38.76"	28'48.36"	*5.7*
7 - Attoyac @ US 59	16076	Attoyac Bayou @ US 59, 2.8 mi NE of US 59 - FM 95 Intersection in Garrison	51'24.14"	27'49.89"	*4.7*
8 - Attoyac @ CR 294	Attoyac 8	Attoyac Bayou @ Nacogdoches CR 294, 2.5 ENE of CR 294 - US 59 Intersection in	50'39.12"	27'8.28"	*2.0*
9 - Attoyac Private 1	Attoyac 9	Attoyac Bayou on Private Property 3 mi E of Garrison	49'40.79"	26'18.96"	*2.8*
10 - Attoyac @ CR 290	Attoyac 10	Attoyac Bayou @ Nacogdoches CR 290, 1.5 mi E of CR 290 - Irving Rd Intersection (0.3 mi N of FM 138 - Irving Rd Intersection)	48'31.68"	25'45.48"	*2.4*
0612 AU_03		***AU_03 lower boundary @ Bear Bayou***			-
0612 AU_02		***AU_02 upper boundary @ Bear Bayou***			*1.2*
11 - Attoyac Private 2	Attoyac 11	Attoyac Bayou on Private Property 4.8 mi SE of Garrison	46'44.04"	25'44.4"	*3.9*
12 - Attoyac @ FM 138	20841	Attoyac Bayou @ FM 138, 6 mi SE of FM 138 - US 59 Intersection in Garrison	46'6.61"	25'34.5"	*1.5*
13 - Attoyac Private 3	Attoyac 13	Attoyac Bayou on Private Property 6.6 mi N of Martinsville	44'20.04"	24'45.36"	*5.1*
14 - Attoyac Private 4	Attoyac 14	Attoyac Bayou on Private Property 5.7 mi N of Martinsville	43'33.6"	24'50.76"	*1.3*
15 - Attoyac @ SH 7	15253	Attoyac Bayou @ SH 7, 1.1 mi E of SH 7 - FM 95 Intersection in Martinsville	38'54.00"	23'50.00"	*10.9*
0612 AU_02		***AU_02 lower boundary @ Polly Branch***			-
0612 AU_01		***AU_01 upper boundary @ Polly Branch***			*6.9*
16 - Attoyac Private 5	Attoyac 16	Attoyac Bayou on Private Property 6.1 mi ESE of Martinsville	35'38.04"	19'36.84"	*13.0*
17 - Attoyac @ CR 392	Attoyac 17	Attoyac Bayou @ Nacogdoches CR 392, 3.4 mi SE of FM 95	32'35.52"	18'23.4"	*7.7*
18 - Attoyac @ SH 21	10636	Attoyac Bayou @ SH 21, 3.1 mi E of FM 95 - SH 21 Intersection near Chireno	30'15.05"	18'13.99"	*6.5*
0612 AU_01		***AU_01 lower boundary @ Granberry Branch***			*8.1*
			Total Stream Length		***88.2***

* site ID denoted with 5-digit number are TCEQ SWQM stations actively monitored in TSSWCB Project 09-10

Table A6.4. Big Iron Ore Creek RUAA Field Survey Sites

Station Name & Map Legend	Site ID*	Site Description	at: 31 ° N ng: 94 ° W		Distance to Previous Site (mi)
	upper extent of waterbody NHD RC 12020005000101				
1 - Big Iron Ore @ US 96	Big Iron Ore 1	Big Iron Ore Creek @ US 96, 5.2 mi N of US 96 - SH 21 Intersection	36'18"	8'54.6"	*2.8*
2 - Big Iron Ore @ FM 3017	Big Iron Ore 2	Big Iron Ore Creek @ San Augustine CR 3017, 1.6 mi N of CR 3017 - FM 711 Intersection	36'46.8"	10'52.68"	*2.7*
3 - Big Iron Ore @ FM 711	Big Iron Ore 3	Big Iron Ore Creek @ FM 711, 10 mi NW of San Augustine	37'12"	14'52.08"	*5.9*
4 - Big Iron Ore @ LR A	Big Iron Ore 4	Big Iron Ore Creek @ LR A, 3.4 mi NNE of LR A - FM 354 Intersection	37'12.91"	14'52.62"	*3.2*
5 - Big Iron Ore @ FM 354	20844	Big Iron Ore Creek @ FM 354, 6 mi N of FM 354 - SH 21 Intersection	33'57.43"	17'22.05"	*3.8*
downstream to Attoyac Bayou confluence; NHD RC 12020005000094					*3.8*
			Total Stream Length		***22.2***

* site ID denoted with 5-digit number are TCEQ SWQM stations actively monitored in TSSWCB Project 09-10

Table A6.5. Naconiche Creek RUAA Field Survey Sites

Station Name & Map Legend	Site ID*	Site Description	at: 31 ° N ng: 94 ° W		Distance to Previous Site (mi)
		upper extent of waterbody NHD RC 12020005000051			
1 - Naconiche @ FM 1087	Naconiche 1	Naconiche Creek @ FM 1087, 8 mi W of FM 1087, FM 95 Intersection	49'43.68"	38'12.12"	*2.0*
2 - Naconiche @ Camp Tonawa	Naconiche 2	Naconiche Creek @ Camp Tonawa Rd in Nacogdoches Co, 2.1 mi S of Camp Tonawa - FM 1087 Intersection	48'19.8"	37'3.72"	*2.2*
** sites 3, 4, 5 evaluated but not included in proposed RUAA sites**					
6 - Naconiche @ US 59	Naconiche 6	Naconiche Creek @ US 59, 8.3 mi NE of US 59 - US 259 Intersection	46'1.2"	33'55.08"	*5.0*
7 - Naconiche @ CR 271	Naconiche 7	Naconiche Creek @ Nacogdoches CR 271, 3 mi S of CR 271 - US 59 Intersection	45'17.64"	32'18.96"	*2.9*
8 - Naconiche @ Cedar Bluff	Naconiche 8	Naconiche Creek @ Cedar Bluff Rd in Nacogdoches Co, 2.7 mi S of Cedar Bluff - FM 2476 Intersection	44'46.98"	31'9.51"	*2.0*
9 - Naconiche @ CR 280	Naconiche 9	Naconiche Creek @ Nacogdoches CR 280, 0.6 mi SW of CR 280 - FM 95 Intersection	44'33.72"	28'44.76"	*4.2*
10 - Naconiche Private 1	Naconiche 10	Naconiche Creek on Private Property, 6.5 mi NNW of Martinsville in Nacogdoches Co	43'37.2"	27'59.04"	*2.2*
11 - Naconiche @ FM 95	20843	Naconiche Creek @ FM 95, 5.6 mi N of FM 95 - SH 7 Intersection	42'43.80"	26'57.86"	*2.2*
		downstream to Attoyac Bayou confluence; NHD RC 12020005000043			*7.1*
			Total Stream Length		***29.8***

* site ID denoted with 5-digit number are TCEQ SWQM stations actively monitored in TSSWCB Project 09-10

Table A6.6. Terrapin Creek RUAA Field Survey Sites

Station Name & Map Legend	Site ID*	Site Description	at: 31 ° N ng: 94 ° W		Distance to Previous Site (mi)
		upper extent of waterbody NHD RC 12020005001348			
0612A		***AU_01 upper boundary of entire water body***	***40'4.43"***	***29'55.81"***	***-***
1 - Terrapin @ CR 234	Terrapin 1	Terrapin Creek @ Nacogdoches CR 234, 4.5 mi SW of CR 234 - FM 95 Intersection	40'4.43"	29'55.81"	*0.3*
2 - Terrapin Private 1	Terrapin 2	Terrapin Creek on Private Property, 1.1 mi WNW of Martinsville in Nacogdoches Co	38'53.52"	25'55.56"	*5.2*
3 - Terrapin @ SH 7	Terrapin 3	Terrapin Creek @ SH 7, 0.6 mi WSW of SH 7 - FM 95 Intersection in Martinsville	38'19.68"	25'24.24"	*1.2*
4 - Terrapin @ FM 95	16084	Terrapin Creek @ FM 95, 0.3 mi S of FM 95 - SH 7 Intersection in Martinsville	38'20.01"	24'53.08"	*0.7*
0612A		***AU_01 lower boundary @ confluence with A***	***38'26.79"***	***23'42.08"***	***2.0***
			Total Stream Length		***9.4***

* site ID denoted with 5-digit number are TCEQ SWQM stations actively monitored in TSSWCB Project 09-10

Table A6.7. Waffelow Creek RUAA Field Survey Sites

Station Name & Map Legend	Site ID	Site Description	at: 31 ° N ng: 94 ° W		Distance to Previous Site (mi)
		upper extent of waterbody NHD RC 12020005000208			
0612B		***AU_02 upper boundary @ headwaters of stream***	***41'44.07"***	***32'42.09"***	-
1 - Waffelow @ FM 1878	Waffelow 1	Waffelow Creek @ FM 1878, 1.5 mi NE of FM 1878 - FM 2112 Intersection	41'44.07"	32'42.09"	*2.5*
2 - Waffelow @ CR 234	Waffelow 2	Waffelow Creek @ Nacogdoches CR 234, 1.6 mi WSW of CR 234 - FM 95 Intersection	41'34.08"	27'54.72"	*5.3*
0612B		***AU_02 lower boundary upstream of unnamed trib .27 km W of CR 234***	***41'36.71"***	***28'3.83"***	-
0612B		***AU_01 upper boundary upstream of unnamed trib .27 km W of CR 234***	***41'36.71"***	***28'3.83"***	***0.2***
3 - Waffelow @ FM 95	16083	Waffelow Creek @ FM 95, 3.9 mi NNW of FM 95 - SH 21 Intersection in Martinsville	41'29.99"	26'16.00"	*2.1*
0612B		***AU_01 lower boundary @ the confluence of Naconiche Creek N of Martinsville***	***41'47.08"***	***25'43.67"***	***2.1***
			Total Stream Length		***12.0***

* site ID denoted with 5-digit number are TCEQ SWQM stations actively monitored in TSSWCB Project 09-10

Table A6.8. West Creek RUAA Field Survey Sites

Station Name & Map Legend	Site ID*	Site Description	at: 31 ° N	ng: 94 ° W	Distance to Previous Site (mi)
		upper extent of waterbody NHD RC 12020005001464			
1 - West @ CR 4054	West 1	West Creek @ Shelby CR 4054, 0.9 mi W of CR 4054 - FM 1645 Intersection	48'54.36"	20'42.72"	*1.3*
2 - West @ CR 4059	West 2	West Creek @ Shelby CR 4059, 0.3 mi W of CR 4059 - FM 1645 Intersection	47'15.36"	20'35.88"	*2.1*
3 - West @ FM 138	West 3	West Creek @ FM 138, 0.35 mi NW of FM 138 - FM 1645 Intersection	45'51.12"	20'44.52"	*2.3*
4 - West @ CR 1510	West 4	West Creek @ Shelby CR 1510, 2.9 mi S of CR 1510 - FM 138 Intersection	43'35.4"	21'47.52"	*4.3*
5 - West @ CR 1546	West 5	West Creek @ Shelby CR 1546, 1 mi E of CR 1546 - CR 1520 Intersection	42'38.52"	22'24.24"	*1.7*
6 - West @ FM 2913	20845	West Creek @ FM 2913, 1.7 mi NW of FM 2913 - SH 7 Intersection	41'13.10"	23'0.09"	*2.7*
		downstream to Attoyac Bayou confluence; NHD RC 12020005000038			*3.6*
			Total Stream Length		***18.0***

* site ID denoted with 5-digit number are TCEQ SWQM stations actively monitored in TSSWCB Project 09-10

BST Analysis

Identification of Sources

New data, of known and specified quality, will be collected and analyzed to differentiate and quantify the relative contributions of livestock, wildlife, and other human and animal *E. coli* sources to Attoyac Bayou and its tributaries. This assessment and differentiation between bacteria sources will utilize the BST Texas Known Source Library coordinated by AgriLife El Paso. The library contains diverse *E. coli* isolates that were selected after screening over 4,400 isolates by genetic fingerprinting to exclude identical isolates from the same sample and include isolates with unique genetic fingerprints. This project will provide sufficient documentation of the data and technical analyses conducted that will aid the project staff in communicating the assessment results to watershed stakeholders and TSSWCB.

100 *E. coli* isolates from 100 different water samples (1 isolate per water sample) collected from across the study area will be analyzed by SAML using the ERIC-PCR and RiboPrinting BST methods described below and compared with isolates from the previously developed Texas Known Source Library. Additionally, 250 water samples collected from each of the monitored stream segments will be analyzed by SAML for *Bacteroidales* PCR markers (general, human, ruminant, swine, equine and others as they become available). An experimental approach flow diagram is presented in Figure A6.4.

SFASU will be responsible for collecting water samples as described earlier in this section and will be responsible for delivering a subset of those samples to the SFASU SPWAL (see Table A6.2 for sampling locations). SFASU SPWAL will be responsible for pre-processing water samples for *E. coli* isolation and *Bacteroidales* PCR. *E. coli* will be isolated from the samples using standard microbiological methods as previously used in TSSWCB and TCEQ BST projects. *E. coli* will be quantified and then isolated from water samples using EPA Method 1603 and modified membrane Thermotolerant *E. coli* (mTEC) medium. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate.

Limited Library Dependant BST

Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), a type of rep-PCR, has moderately high ability to resolve different closely related bacterial strains (Versalovic et al. 1994). Consumable costs for ERIC-PCR are inexpensive and labor costs for sample processing and data analyses are moderate. ERIC-PCR is a genetic fingerprinting method used in previous BST studies as well as many microbial ecology and epidemiological studies. ERIC elements are repeat DNA sequences found in varying numbers and locations in the genomes of different bacteria such as *E. coli*. The PCR is used to amplify the DNA regions between adjacent ERIC elements. This generates a DNA banding pattern or fingerprint which looks similar to a barcode pattern. Different strains of *E. coli* bacteria have different numbers and locations of ERIC elements in their bacterial genomes, and therefore, have different ERIC-PCR fingerprints. ERIC-PCR is useful as a screening technique for library development because of its moderate cost and moderately high ability to resolve different strains of the same species of bacteria. Though rep-PCR banding patterns for isolates tend to be generally stable, differences in fingerprint image processing and PCR protocols between laboratories may result in reduced between-laboratory reproducibility and pose a challenge to generating a composite library in multiple laboratories. Rigorous QA/QC, standardized protocols for PCR and image processing, and adequate training of personnel is crucial for generation of comparable data (Jones et al. 2009).

Ribotyping is a genetic fingerprinting method used in previous BST studies and many microbial ecology and epidemiological studies. In general, an endonuclease enzyme (*Hind* III) selectively cuts *E. coli* DNA wherever it recognizes a specific DNA sequence. The resulting DNA fragments are separated by size and probed for fragments containing particular conserved ribosomal RNA gene sequences, which results in DNA banding patterns or fingerprints that look similar to barcodes. Different strains of *E. coli* bacteria have differences in their DNA sequences and different numbers and locations of enzyme cutting sites, and therefore have different ribotyping fingerprints. The DuPont Qualicon RiboPrinter Microbial Characterization System allows automation of the ribotyping ('RiboPrinting').

A total of 100 *E. coli* isolates obtained from ambient water samples from across the study area will be characterized using ERIC-PCR and RiboPrinting. DNA patterns of those isolates will be compared to the Texas Known Source Library of *E. coli* isolates from known animal and human sources collected throughout Texas. Water isolates will be identified to cattle, other livestock, avian and non-avian wildlife, domestic sewage, and pet sources (six-way split), as well as a broader three-way split of livestock, domestic sewage and wildlife.

Library Independent BST

PCR genetic testing for *Bacteroides* fecal bacteria will be performed by SAML to determine the source of the fecal pollution. The *Bacteroidales* PCR method is a culture-independent molecular method which targets genetic markers of *Bacteroidales* and *Prevotella* spp. fecal bacteria that are specific to humans, ruminants (including cattle and deer), pigs, and horses (Bernhard& Field 2000; Dick et al. 2005). The method has high specificity and moderate sensitivity (Field et al. 2003). 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. For pre-processing of water samples for *Bacteroidales* PCR, SAML will filter the water samples, place the filters in DNA lysis buffer and freeze at -80° C until analysis. At the time of analysis, SAML will extract and purify DNA from the filters. DNA extracted from the water samples will be tested for the general, human, ruminant (including cattle and deer), pig (including feral hogs), and horse fecal markers. Results are typically expressed as presence/absence of the host-specific genetic markers; therefore, this method is not quantitative.

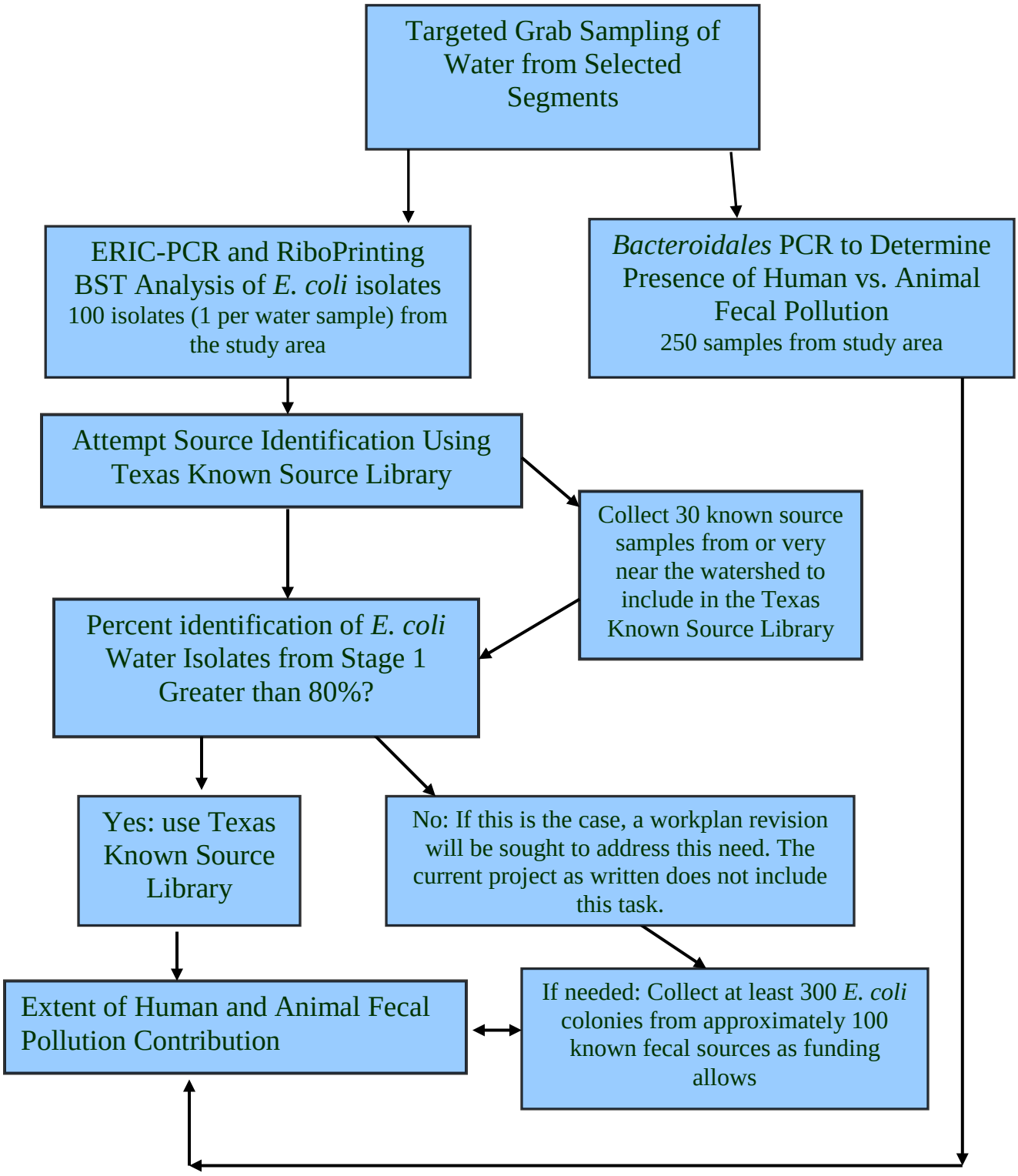


Figure A6.4. Flow Diagram of Experimental Approach for BST

Section A7: Quality Objectives and Criteria for Data Quality

The objectives for this project are as follows:

1) Develop and obtain approval for a QAPP
2) Classify current land use / land cover for the Attoyac Bayou watershed for use in computer based modeling and WPP development.
3) Collect environmental and water quality data to support the development of a WPP
4) Utilize computer based programming to develop LDCs and evaluate contaminant loading potential using the SELECT model
5) Utilize BST as a means to help direct bacteria targeted management measures that will be outline in the WPP

Land Use/Land Cover Update

A combination of satellite based image classification schemes and where needed "heads-up digitizing" of the 2004 and 2009 National Agriculture Imagery Program (NAIP) aerial photos of the area in ESRI's ArcGIS 9.x software will be used to classify the current land use / land cover. NAIP provides two main products: 1 meter ground sample distance (GSD) ortho imagery rectified to a horizontal accuracy of within +/- 3 meters of reference digital ortho quarter quads (DOQQs) from the National Digital Orthophoto Program (NDOP) (2004 imagery); and, 2 meter GSD ortho imagery rectified to within +/- 20 meters of reference DOQQs (2005 imagery). The tiling format of NAIP imagery is based on a 3.75' x 3.75' quarter quadrangle with a 360 meter buffer on all four sides. NAIP quarter quads are rectified to the Universal Transverse Mercator (UTM) coordinate system, North American Datum (NAD) 83 and cast into a single predetermined UTM zone.

As a point of comparison, USGS National Land Cover Data (NLCD) 2001 data is created with Landsat Thematic Mapper images. Each image is precision terrain-corrected using 3-arc-second digital terrain elevation data (DTED), and georegistered using ground control points. The resulting root mean square registration error is less than 1 pixel, or 30 meters. This data will be used as a cross check to evaluate the accuracy of the LULC assessment.

To achieve the needed precision and accuracy, the land use / land cover classification scheme to be used in this delineation will include at a minimum the twelve classifications discussed in A6. Individual LULC classes will be identified and delineated with a minimum mapping unit of 2 acres on screen.

Representativeness will be addressed by collecting ground control points for at least ten locations per land use type per watershed. This GPS survey will utilize the Garmin GPS 72 Global Positioning System Receiver in the WGS84 (World Geodetic System of 1984) Mode to obtain control point latitude/longitude values within 20 feet of true locations at the 95% confidence level. This level of accuracy is consistent with Tier 3 described in the EPA National Geospatial Data Policy. The Garmin GPS 72 will be set to capture data provided that at least four satellites are in view and the Position Dilution of Precision (PDOP) value remains at 6 or below. The receiver will be set to provide audible or visual warnings when the quality

settings are exceeded. Sample interval and time on station will be consistent with Garmin GPS 72 Manual recommendations. Post-processing the GPS data will be accomplished using the vendor's software package operating on a local workstation. The higher end software package will perform statistical analyses on the point data downloaded from the GPS receiver. For 20 feet data accuracy, any data points with a standard deviation of 6 feet or more will be a basis to exclude that data point from the collection. Ideally, the standard deviation for 20-foot accuracy data should be 2 feet or less at the 95% confidence level.

Once the ground control points are collected as outlined in the previous paragraph, the individual LULC classes will be verified through comparison with the ground control points to ensure an accuracy of 80% or greater. This will be complemented with aerial photographs and other ancillary data described in Section B4. Comparability will be addressed by collecting, analyzing, and reporting the data as described in section B5 of this document.

A completeness goal of 100% is needed for the project. Valid data is required for each land use / land cover class mapped in order to complete the cover maps for each watershed.

Surface Water Quality Monitoring

The objective of this section is to ensure that data collected meets the data quality objectives (DQOs) of the project. One objective is to identify specific sources of bacteria and ammonia entering the Attoyac Bayou. A second objective is to monitor micro-watersheds through data collection and analysis, and provide data to inform soil and water conservation districts (SWCD's), stakeholder committee, and landowners of any potential or existing water quality issues and/or problems. Achievement of these objectives will support decisions for implementation of appropriate best management practices (BMPs) in order to reduce fecal bacteria levels in the Attoyac Bayou watershed to comply with existing water quality standards.

Following are actions that will be undertaken by this project to assess bacterial pollution within the Attoyac Bayou Watershed:

- Monitor water quality as related to bacterial pollution in Attoyac Bayou and designated tributaries by in-stream water sampling
- Determine the source of the bacterial impairment using BST

The measurement performance criteria to support the project objective are specified in Table A.7-1.

Consistent with the most recent version of TCEQ's *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods* (TCEQ SOP, V1), routine grab samples will be collected on a bi-weekly (twice monthly) basis. During routine sampling measurements of DO, conductivity, pH, salinity, stream flow, and water temperature will be obtained *in situ*. These data will be logged on field data sheets, incorporated into a computer based database maintained by SFASU and transmitted to ANRA for inclusion in the master database that they will maintain.

Water samples collected will be transported to the ANRA for nutrient analysis, bacteria enumeration and data logging. SFASU will deliver water samples to ANRA within designated holding times for respective analysis; ANRA will use designated methods outlined in Tables A7.1 and B2.1. Appropriate DQOs and QA/QC requirements for this analysis are also reported in Tables A7.1 and B2.1.

Additionally, the SFASU SPWAL will receive and pre-process water and known source fecal samples for later BST processing. SFASU WET Lab will use designated methods outlined in Tables A7.1 and B2.1. Appropriate DQOs and QA/QC requirements for this analysis are also reported in Tables A7.1 and B2.1.

LDC and SELECT Data Analysis

Faculty in the BAEN Department at TAMU will conduct a phased modeling effort to develop pollutant source and loading information and estimates of needed. The objectives of the water quality modeling portion of this project are as follows:

1) Develop LDCs on currently available ammonia and bacteria data for each monitoring site on the Attoyac Bayou. LDCs developed will be consistent with *An Approach for Using Load Duration Curves in the Development of TMDLs* (EPA 2007a), *Options for Expressing Daily Loads in TMDLs* (EPA 2007b), and *.Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality* (EPA 2008).
2) Update LDCs developed using historic water quality data with water quality data collected under Task 5. LDCs will be used to estimate needed load reduction for ammonia and bacteria at each monitoring site in the waterbody.
3) Conduct watershed modeling using the SELECT approach for the Attoyac Bayou. Information collected in Tasks 4, 5, 7 and 8 as described in the project work plan will be incorporated with information from LDC analyses to estimate pollutant loadings from various sources within the watershed and identify potentially critical loading areas.

SELECT – this approach is being developed by SSL and BAEN. It is similar to the BSCL (Zeckoski, et al. 2005) that is used in TMDL development. High quality spatial data (LU/LC data developed under Task 4 of this project, soil survey geographic (SSURGO) soils data, NHD, etc) will be processed and utilized in SELECT approach. Distributions for input parameters for SELECT will be created based on literature values and expert knowledge.

LDC – this approach has been utilized in several TMDL projects as an initial screening-tool to evaluate the actual temporal load trends in streams (Cleland, 2003; Stiles, 2001). In cases of violations, it is necessary to determine the required load-reduction in that region near the monitoring station. The load-reductions should be calculated for all flow-regimes of the stream. In order to do this continuous monitoring data will be simulated using the actual monitoring data by regression methods. Uncertainty of the model will be estimated via

residual error analysis. The straight line passing through residual error plot should have a slope of zero.

RUAA

The objective of this portion of the project is to assess contact recreation uses and support watershed planning for the Attoyac Bayou and its tributaries by conducting a RUAA. This process will evaluate the physical and hydrological characteristics of assessed waterbodies and will combine these data with interviews and historical assessments to gain a true idea of actual waterbody uses.

BST Analysis

The objective of this portion of the project is to assess contact recreation use impairments and support watershed planning for the Attoyac Bayou and its tributaries by conducting BST. The measurement performance specifications to support the project objective are specified in Table A7.2. Laboratory measurement QC requirements and acceptability criteria are provided in Section B5.

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 the program-defined reporting specifications for each analyte and yield data acceptable for the TCEQ's water quality assessment. A full listing of AWRLs can be found at http://www.tceq.state.tx.us/compliance/monitoring/crp/qa/index.html. The limit of quantitation (LOQ) 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 following requirements must be met in order to report results to the CRP:

- **The laboratory's 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 for each analytical batch of CRP Samples analyzed.**

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

Precision

Precision is the degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. It is a measure of agreement among replicate measurements of the same property, under prescribed similar conditions, and is an indication of random error.

Field splits are used to assess the variability of sample handling, preservation, and storage, as well as the analytical process, and are prepared by splitting samples in the field. Control limits for field splits are defined in Section B5.

Laboratory precision is assessed by comparing replicate analyses of laboratory control samples in the sample matrix (e.g. deionized water, sand, commercially available tissue) or sample/duplicate pairs in the case of bacterial analysis. Precision results are compared against measurement performance specifications and used during evaluation of analytical performance. Program-defined measurement performance specifications for precision are defined in Table A7.1.

Bias

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

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.

Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions and recreational uses. The representativeness of the data is dependent on the sampling locations, the conditions under which surveys are performed, and the survey procedures. According to TCEQ guidance, the RUAA field surveys would ideally be performed at a frequency of three sites per five stream miles. This would assure maximum capture of stream recreational uses. Additionally, sites will be surveyed preferentially during high recreational use potential, both temporally and hydrologically. The final determination of the applicability of individual and collective site recreational use conditions will be made in the Technical Report.

Comparability

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

Table A7.1. Measurement Performance Specifications

Parameter	Units	Matrix	Method	Parameter Code	AWRL	Limit of Quantitation (LOQ)	Precision (RPD of LCS/LCSD)	Bias % Rec. of LCS	LOQ Check Standard % Rec	Lab
Field Parameters										
pH	pH/units	water	EPA 150.1 & TCEQ SOP, V1	00400	NA	NA	NA	NA	NA	Field
DO	mg/L	water	SM 4500-O G & TCEQ SOP, V1	00300	NA	NA	NA	NA	NA	Field
Specific Conductance	µS/cm	water	EPA 120.1 & TCEQ SOP, V1	00094	NA	NA	NA	NA	NA	Field
Temperature	Celcius	water	SM 2550 B & TCEQ SOP, V1	00010	NA	NA	NA	NA	NA	Field
	Celcius	air	EPA 170.1 & TCEQ SOP, V1	00020	NA	NA	NA	NA	NA	Field
Days since last significant rainfall	days	NA	TCEQ SOP, V1	72053	NA	NA	NA	NA	NA	Field
Flow	cfs	water	TCEQ SOP, V1	00061	NA	NA	NA	NA	NA	Field
Total water depth	meters	water	TCEQ SOP, V1	82903	NA	NA	NA	NA	NA	Field
Flow measurement method	1-gage 2-electric 3-mechanical 4-weir/flume 5-doppler	water	TCEQ SOP, V1	89835	NA	NA	NA	NA	NA	Field
Continuous flow measurement (automated sampler)	5-doppler	water	NA	NA	NA	NA	NA	NA	NA	Field
Flow severity	1-no flow 2-low 3-normal 4-flood 5-high 6-dry	water	TCEQ SOP, V1	01351	NA	NA	NA	NA	NA	Field
Present weather	1-clear 2-partly cloudy 3-cloudy 4-rain 5-other	NA	TCEQ SOP, V1	89966	NA	NA	NA	NA	NA	Field
Conventional and Bacteriological Parameters ANRA & SFASU WET Lab										
TSS	mg/L	water	SM 2540 D	00530	4	2.5 / 1	20	80-120	NA	ANRA
Ammonia-N, total	mg/L	water	SM 4500NH3-C or D	00610	0.1	0.1	20	80-120	70-130	ANRA
Nitrate/Nitrite-N	mg/L	water	SM 4500NO3-E	00630	0.05	0.04	20	80-120	70-130	ANRA
Dissolved Ortho-P	mg/L	water	SM 4500-PE	70507	0.04	0.04	20	80-120	70-130	ANRA
Total P	mg/L	water	SM 4500-PE	00665	0.06	0.06	20	80-120	70-130	ANRA
E. coli, IDEXX	MPN/100mL	water	SM 9223-B	31699	1	1	0.5**	NA	NA	ANRA
E. coli, mTEC	cfu/100mL	water	EPA 1603	31648	1	1	3.27* ΣRlog/n	NA	NA	SFSAU WET

** Based on a range statistic as described in Standard Methods, 20th Edition, Section 9020-B, AQuality Assurance/Quality Control – Intralaboratory Quality Control Guidelines. This criterion applies to bacteriological duplicates with concentrations >10 MPN/100mL or >10 organisms/100mL.
EPA = Methods for Chemical Analysis of Water and Wastes, March 1983
SM = Standard Methods for Examination of Water and Wastewater, 21st edition
TCEQ SOP, V1 = TCEQ's Surface Water Quality Monitoring Procedures, Volume 1

Table A7.2. Measurement Performance Specifications for BST Analysis

Parameter	Method Type	Method	Method Description	Precision of Laboratory Duplicates*	Bias*	Percent Complete **	Lab
E. coli RiboPrinting	DNA/image matching	EP AREC SOP	RiboPrinting	90% identical	90% correct	90	SAML
E. coli ERIC-PCR	DNA/image matching	EP AREC SOP	ERIC-PCR	90% identical	90% correct	90	SAML
Bacteriodales PCR	PCR presence / absence	EP AREC SOP	*Bacteriodales* PCR	100% agreement	90% correct	90	SAML
E. coli isolation	membrane filter culture on modified mTEC agar	USEPA 1603	Membrane Filter	NA	NA	NA	SFASU

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.

Section A8: Special Training Requirements/Certification

Land Use/Land Cover Update

No special certifications are required. However, all personnel involved in classification of land use and land cover will have the appropriate education and training required to adequately perform their duties. GIS technicians will be experienced or trained in using Garmin GPS 72 GPS Receivers, (ESRI) ARCINFO and ARCVIEW.

Surface Water Quality Monitoring

All personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. SFASU personnel involved in this project have been trained in the appropriate use of field equipment, laboratory equipment, laboratory safety, cryogenics safety, and all applicable procedures outlined in the TCEQ SOP V1.

LDC and SELECT Data Analysis

All BAEN personnel involved in model calibration, validation, and development will have the appropriate education and training required to adequately perform their duties. No special certifications are required.

RUAA

No special training required. All personnel involved have received instruction on proper methods for collecting physical and hydrological information on assessed waterbodies. No special certifications are required.

BST Analysis

All personnel involved in sample analyses and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. SAML personnel involved in this project have been trained in the appropriate use of laboratory equipment, laboratory safety, cryogenics safety, and all applicable El Paso AgriLife Research and Extension Center (EP AREC) SOPs. 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. Finally, SAML is NELAP-certified for enumerating *E. coli* in both non-potable and drinking water using USEPA Method 1603.

Section A9: Documentation and Records

Land Use/Land Cover Update

Digital files of land cover data for each watershed will be produced in shapefile or ArcGIS grid format and stored on CD-ROM disks. Multi-color hard copy maps of land cover can be produced at various geographic scales from these digital files. CES with assistance from TWRI will produce a hard copy land cover map the watersheds. Other products will be produced as required by the TSSWCB, cooperators and other data users.

Metadata documentation will be developed and will document data sources, processing techniques, accuracy assessment, and other pertinent information.

Appendix B represents the field data collection form used for this project. Other records and documentation to be developed for this project include the following: digital files of spatial data, field data, and scanned photographs.

Records of field data, original aerial photos, digital files used for classifying LULC and accuracy assessment, and corrective action reports (CARs) (Appendix A) will be maintained and archived by CES for at least five years.

Surface Water Quality Monitoring

Hard copies of general maintenance records, all field data sheets, chain of custody (COC) forms, laboratory data entry sheets, calibration logs, and corrective action reports (CARs) will be archived by each laboratory for at least five years. In addition, SFASU and ANRA will archive electronic forms of all project data for at least five years. 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 D, and blank field data reporting forms are presented in Appendix C.

LDC and SELECT Data Analysis

All records, including modeler's notebooks and electronic files, will be archived by BAEN for at least five years. These records will document model testing, calibration, and evaluation and will include documentation of written rationale for selection of models, record of code verification (hand-calculation checks, comparison to other models), source of historical data, and source of new theory, calibration and sensitivity analyses results, and documentation of adjustments to parameter values due to calibration. Electronic data on the project computers and the network server are backed up daily to a tape drive. In the event of a catastrophic systems failure, the tapes can be used to restore the data in less than one day's time. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

RUAA

A RUAA report will be compiled in accordance with the TCEQ *Recreational Use-Attainability Analyses (RUAAs) – Procedures for a Comprehensive RUAA and a Basic RUAA Survey* (TCEQ 2009).

RUAA forms will include:

- Information to be collected shall satisfy those questions found in the Contact Information Form (Appendix H)
- Field data sheets, Interview Forms, and RUAA Summary in electronic format
- Digital photographic record, cataloged in an appropriate manner

RUAA related data collected by SFASU will be stored in paper and electronic format. Surveys conducted will be initially logged in paper format but will be digitized at a later date and stored on personal computers that are routinely backed up on external storage media.

BST Analysis

Individual laboratory notebooks, which contain printouts of laboratory data and hand written observations and data, are kept by individual analysts at SAML or the SCSC Project Co-Lead for at least five years. When lab notebooks are filled, they are stored for at least five years by the SCSC Project Co-Lead/Laboratory Manager in hardcopy form. The SAML keeps electronic data on personal computers for the duration of the project and then in hardcopy files for 5 years after the project. COCs and attached documents are stored in numerical order in three-ring binders in the SCSC Project Co-Lead/Laboratory Manager's office for at least five years. In addition, the SCSC Project Co-Lead/Laboratory Manager will archive electronic forms of all project data for at least five years on personal computers and fire-resistant cabinets. Lab data reports from the SAML, as included in the final report, and other reports as required, will report test results clearly and accurately.

Combined Project Documentation

Quarterly progress reports disseminated to the individuals listed in section A3 will note activities conducted in connection with the water quality modeling project, items or areas identified as potential problems, and any variations or supplements to the QAPP. Final reports on the SELECT modeling analysis and the LDC analysis will be developed as chapters to the WPP. Outcomes will be submitted to the established stakeholder group and utilized in future WPP development.

CARs will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference at TWRI and will be disseminated to the individuals listed in section A3. CARs resulting in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in updates or amendments to the QAPP.

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 is presented in Appendix A and a blank COC form is presented in Appendix D.

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

Table A9.1 Project Documents and Records

Document / Record	Location	Retention	Form
QAPP, amendments and appendices	TWRI	5 years	Paper or Electronic
Chain Of Custody records	ANRA, SAML, SFASU	5 years	Paper or Electronic
Field data, aerial imagery & digital data used for LU/LC classification	CES	5 years	Paper or Electronic
Modeler's notebooks & electronic files	BAEN	5 years	Paper or Electronic
Field notebooks & data sheets	SFASU	5 years	Paper or Electronic
Field equipment calibration & maintenance logs	SFASU	5 years	Paper or Electronic
Corrective Action Report	TWRI	5 years	Paper or Electronic
Bacteriological data log sheet	ANRA, SAML, SFASU	5 years	Paper or Electronic
Laboratory QA Manuals	ANRA, SAML, SFASU	5 years	Paper or Electronic
Laboratory methods guidance	ANRA, SAML, SFASU	5 years	Paper or Electronic
Instrument raw data files, readings & printouts	ANRA, SAML, SFASU	5 years	Paper or Electronic
Lab equipment calibration records & maintenance logs	ANRA, SAML, SFASU	5 years	Paper or Electronic
Lab data reports	TWRI / TSSWCB	3 years	Paper or Electronic
Progress reports / final report / data	TWRI / TSSWCB	3 years	Paper or Electronic

Data Transfer between Entities

Data transfer between entities occurs via electronic means. Specific format of the data transferred depends on the specific data and includes ArcView, MS Office, and PDF formats.

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 can be accomplished by submitting a cover letter stating the status of the QAPP and a copy of new, signed approval pages for the QAPP.

Amendments to the QAPP may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and non-conformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances. Requests or amendments are directed from the TWRI Project Lead to the TSSWCB PM in writing. The changes are effective immediately upon approval by the TSSWCB PM and QAO, or their

designees, and the EPA Project Officer. Amendments to the QAPP and the reasons for the changes will be documented, and copies of the approved QAPP Expedited Amendment form will be 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.

Section B1: Sampling Process Design (Experimental Design)

Land Use/Land Cover Update

The production of a land cover map is an iterative process based on data from satellite imagery, aerial photography, existing maps and field reconnaissance. NAIP satellite imagery from 2004 and 2009 has been obtained and will be paired with ground-truthed field data. Land use / land cover will be assigned to twelve categories according to the category descriptions provided in Section A6.

Ground reference data must be collected to train the computer software to recognize the spectral reflectance of various land cover categories represented in the NAIP imagery. Since ground reference data generally cannot be collected for the entire project area, representative samples will be used.

CES staff will attempt to collect or acquire at least ten actual ground locations per land use throughout the watershed for use in mapping land cover. These locations will be used to conduct supervised classifications of remote sensing data from satellite imagery. This data will also be used for accuracy assessment as outlined in Section B5.

Field data will be collected according to standard protocols. CES will review field data and assign appropriate classification prior to digitizing the data for GIS analysis. Descriptions of land use / land cover that cannot be assigned a class corresponding to the scheme used in labeling classes on the land cover map will be rejected.

Types and numbers of samples required: CES will acquire 10 representative ground locations for each land cover class labeled on the land cover map.

Sampling Locations and frequencies: CES has a goal of 120 field sites across the watershed with a minimum of 10 sites for each land use / land cover class.

Surface Water Quality Monitoring

Data collection and analysis will play a pivotal role in this project and will provide data to inform SWCDs and landowners of any potential or existing water quality issues and/or problems and form the foundation for developing the Attoyac Bayou WPP. In addition, water samples will be analyzed to determine the source of bacteria entering the stream. This information will be instrumental in evaluating potential BMPs to implement in the watershed as well as aid in WPP development. Achievement of these objectives will support decisions on how to best target management measures to reduce fecal bacteria levels in the Attoyac Bayou watershed. The waterborne constituents that will be measured are shown in Table B1.1.

The sampling program is designed to characterize water quality of both base and high flow conditions in the Attoyac Bayou and its tributaries. Water quality grab samples will be collected on bi-weekly (twice monthly) intervals for all constituents as directed by TCEQ

SOP, V1. Sampling types, frequencies and expected numbers are described in Table B1.2. Physical parameters that will be measured *in situ* during routine sampling and include flow (cfs), specific conductance, DO, pH, salinity, and water temperature; other noted items will include the flow severity, days since last significant rainfall and present weather conditions. Sites that are dry or with pooled water will not be sampled and conditions will be noted on the field data sheet. Water quality samples collected as part of the routine sampling schedule will be analyzed for bacteria and nutrients as outlined in Table A7.1. Additional water samples and field blanks will be collected and delivered to SFASU SPWAL for *E. coli* analysis and preparation for future BST analysis.

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; this is left up to the discretion of the sampling crew.

To evaluate the influences of elevated flows on instream bacteria levels, samples will be collected with automated sampling devices. These devices are equipped with continuous depth and velocity monitoring instrumentation that will both trigger the collection of samples and calculate streamflow. Once activated, field staff are notified and water samples are retrieved within prescribed holding times.

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

Collection of fecal material samples from known sources will also be done and will be used to validate the BST methodologies. Approximately thirty known source samples will be collected throughout the course of the project and will include domestic animals, wildlife and human sources. These known sources of bacteria (domestic animals, wildlife and humans) will not be collected from the same locations during every collection due to the nature of the animals. Human sources are from specific areas, but will be selected based on cooperation of the individuals. Therefore; specific global positioning system (GPS) coordinates cannot be listed for sample collections of this nature.

LDC and SELECT Data Analysis

Not relevant.

Table B1.1: Waterborne Constituents Evaluated

Parameter	Reporting Units	Status
Field Parameters		
pH	pH/units	non-critical
DO	mg/L	non-critical
Specific Conductance	µS/cm	non-critical
Temperature	degrees Celcius	non-critical
Days since last significant rainfall	days	non-critical
Flow	cfs	critical
Water depth	meters	critical
Flow severity	1-no flow, 2-low, 3-normal, 4-flood, 5-high, 6-dry	critical
Present weather	1-clear, 2-partly cloudy, 3-cloudy, 4-rain, 5-other	non-critical
Laoratory Analysis		
TSS	mg/L	non-critical
Ammonia-N, total	mg/L	critical
Nitrate/Nitrite-N	mg/L	non-critical
Dissolved Ortho-P	mg/L	non-critical
Total P	mg/L	non-critical
E. coli, IDEXX	MPN/100mL	critical
E. coli, mTEC	cfu/100mL	critical

RUAA

CES and SFASU will collect information that can be used to evaluate recreational uses in the waterbodies in the study area. Methods used and sampling process design shall be consistent with the TCEQ *Procedures for a Comprehensive RUAA and a Basic RUAA Survey*, (TCEQ 2009). CES and SFASU will conduct field surveys at selected sites during the period people would most likely be using the waterbody for contact recreation; surveys shall ascertain the suitability of the streams for contact recreation use and shall document the hydrological characteristics of the stream.

BST Analysis

To provide sufficient water quality data to characterize bacteria loadings across the various flow regimes, routine ambient monitoring will be conducted by SFASU once every 2 weeks at 10 stream sites (see Table A6.2). Additionally, 4 identified point source discharges in the watershed will be sampled quarterly for five quarters for a total of 20 samples. SFASU with cooperation from ANRA will ensure that permission is obtained from both the TCEQ and the respective entities to monitor these point source discharges. This data will provide information that will allow for an estimate of possible contributions from wastewater discharges. SFASU will conduct biased-flow monitoring under high flow (storm event) conditions at 2 sampling sites during at least 10 storm events utilizing automated monitoring equipment. Field data and samples will be collected following procedures detailed in the *TCEQ SWQM Procedures, Volume 1 (RG-415).*

Samples collected by SFASU will be delivered to the lab at ANRA for processing and analysis; a secondary set of water samples collected will be delivered to SFASU SPWAL for BST pre-processing. SFASU SPWAL will process and store this subset of collected water samples and will arrange for shipment or delivery to SAML for BST analysis (Table B1.2). SAML will perform Bacteroidales PCR on approximately 250 individual water samples collected by SFASU between July 2010 and May 2012. The samples will include: 1) 21 sample events for each of the 10 stream sites; 2) 5 sample events for each of the 4 point source discharges; and 3) 10 sample events for each of the 2 monitored stream sites during and following storm events. SAML will also isolate and fingerprint (ERIC-RP) *E. coli* (one per site per sample event) for: 1) 8 sampling events at the 10 stream sites, 2) 2 sampling events at the 4 point source discharges and 3) 6 storm sampling events at the two storm monitoring stations. This results in a total of 100 individual samples analyzed using ERIC-PCR. Approximate sample collection timing is outlined in Table B1.2; weather conditions will dictate precisely when samples will be collected.

Table B1.2. Samples to be Analyzed using *Bacteroidales* PCR and ERIC-RP

X' denotes a single sampling event* Parameter			*Bacteriodales*	*Stream: 10 sites*	*Point Sources (4)*	*Storm Samples: 2 sites***	*E. coli (ERIC-RP)*	*Stream: 10 sites*	*Point Sources (4)*	*Storm Samples: 2 sites*	Total Number of Samples
Year	Project Month	Quarter									
May '10	Month 7	Q 3									0
June '10	Month 8										0
July '10	Month 9	Q 4									0
Aug '10	Month 10			X		X		X		X	22
Sep '10	Month 11			X							10
Oct '10	Month 12	Q 5		X							10
Nov '10	Month 13			X	X	X		X		X	28
Dec '10	Month 14			X							10
Jan '11	Month 15	Q 6		X							10
Feb '11	Month 16			X	X	X		X	X	X	32
Mar '11	Month 17			X							10
Apr '11	Month 18	Q 7		X							10
May '11	Month 19			X	X	XX		X		X	30
June '11	Month 20			X							10
July '11	Month 21	Q 8		X							10
Aug '11	Month 22			X	X	XX		X	X	X	36
Sep '11	Month 23			X							10
Oct '11	Month 24	Q 8		X							10
Nov '11	Month 25			X		X		X		X	28
Dec '11	Month 26			X							10
Jan '12	Month 27	Q 9		X							10
Feb '12	Month 28			X	X	X		X			22
Mar '12	Month 29			X							10
Apr '12	Month 30	Q 10		X							12
May '12	Month 31					X		X			10
	Total Number of Samples		250	210	20	20	100	80	8	12	350

* An 'X' denotes one complete subset (1 sample collected from each site) of samples collected to be analyzed for respective BST analysis

** Approximately one storm event sample will be analyzed per site per quarter using Bacteroidales PCR and every other quarter using ERIC-RP. Storm sampling timeframe may also vary depending on the timing of run-off producing storms

Section B2: Sampling Method Requirements

Land Use/Land Cover Update

Phase 1 Acquisition:

Ancillary data will be used to classify the satellite based images into classes. CES will be using existing NAIP 2004 and 2009 aerial imagery and collected field data from the Natural Resource Conservation Service (NRCS) as sources to define LULC polygons. The geographic location of the polygons is known and is matched to the same location on the imagery.

Phase 2 Acquisition:

Field sampling will be used to verify individual LULC classes identified and delineated. Ground control points used in the field sampling will be collected for at least ten locations per land use type per using GPS units with an accuracy of 1-20 ft.

LULC categories are identified in the field by an observer who is knowledgeable about LULC identification and classification standards. Observed LULC classifications are recorded on data forms provided by the CES (Appendix B). No specialized equipment is used to collect the sample data.

Ancillary data will be used to supplement the sample data gathered by the field personnel. These sources include color infrared, black and white and color aerial photography of the same time period as the imagery and other sources that become available during the classification process. This includes 2001 NLCD data will be used as a cross reference to verify the locations of land use polygons.

Documentation of Field Sampling Activities

Field sampling activities are documented in field notebooks. Site identification, date, time, personnel, and conditions at the site are recorded for every sampling event.

Recording Data

All CES personnel will follow the basic rules for recording information including: (1) writing legibly in indelible, waterproof ink with no modifications, write-overs or cross-outs; (2) correcting errors with a single line followed by an initial and date; and (3) closing-out incomplete pages with an initialed and dated diagonal line.

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

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.

Surface Water Quality Monitoring

Field Sampling Procedures

Field sampling will be conducted according to procedures documented in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue, 2012 (RG-415)*. Additional aspects outlined in Section B below reflect specific requirements for sampling. Sampling will be done so that it is consistent with sampling conducted under the guise of the Clean Rivers Program. Field sampling activities are documented on field data reporting forms as presented in Appendix C.

All sample information will be logged into a field log. The following will be recorded for all water sampling:

- station ID
- location
- sampling time
- date
- water depth
- flow rate
- sample collector's name/signature

Detailed observational data are recorded including water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, days since last significant rainfall, estimated hours since rainfall began (if applicable), and flow severity.

Typically, water samples will be collected directly from the stream (midway in the stream channel) into sterile wide-mouthed polypropylene bottles or bags. Water samples used for *E. coli* analysis will be collected in sterile bags, those undergoing the IDEXX method will be collected in sterile polyethylene bottles provided by ANRA, and samples undergoing the mTEC method will be collected in sterile 125 mL Whirl-Pak bags. All sample containers will be labeled with the following information:

- collection date
- collection time
- sample location
- and sampler's initials

Care will be exercised to avoid the surface microlayer of water, which may be enriched with bacteria and not representative of the water column. In cases where, for safety reasons, it is inadvisable to enter the stream bed, and boat access is not practical, staff will use a clean bucket and rope from a bridge to collect the samples from the stream. If a bucket is used, care will be taken to avoid contaminating the sample. Specifically, technicians must exert care to ensure that the bucket and rope do not come into contact with the bridge. The bucket must be

thoroughly rinsed between stations. Buckets are also to be sanitized between sampling stations with a bleach- or isopropyl alcohol-soaked wipe. The first bucketful of water collected from a bridge is used to rinse the bucket. Rinse water is not returned to the stream, but is instead disposed of away from the sampling site to ensure that the collected sample will not be affected by the bleach or alcohol residual. Samples are collected from subsequent buckets of water. This type of sampling will be noted in the field records.

Water temperature, pH, specific conductivity, specific conductance, and DO will be measured and recorded *in situ* with a multiprobe whenever samples are collected. Flow is measured with an electronic flow meter as described in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue, 2012 (RG-415).* All field measurements will be conducted in accordance with the methods listed in Table B.4-1. All samples will be transported in an iced container to the laboratory for analysis.

Table B2.1. Storage, Preservation and Handling Requirements

Parameter	Matrix	Container	Preservation	Temp	Sample Size	Holding Time
TSS	water	PC HDPE	n/a	< 6°C	1000 mL	7 days
Ammonia-N, total	water	PC HDPE	Acidify w/ H2SO4 to pH ,2	< 6°C	1000 mL	28 days
Nitrate/Nitrite-N	water	PC HDPE	Acidify w/ H2SO4 to pH ,2	< 6°C	500 mL	28 days
Dissolved Ortho-P	water	PC HDPE	filter in field (<15 minutes)	< 6°C	150 mL	48 hours
Total P	water	PC HDPE	Acidify w/ H2SO4 to pH ,2	< 6°C	250 mL	28 days
E. coli, IDEXX	water (routine)	sterile PE	n/a	4°C	200 mL	6 hours
E. coli, mTec	water (routine)	SSB	n/a	4°C	150 mL	6 hours
E. coli, IDEXX	water (storm)	sterile PE	n/a	<10°C	200 mL	24 hours
E. coli, mTec	water (storm)	SSB	n/a	<10°C	150 mL	24 hours
Fecal specimen	feces	sterile container	n/a	4°C	30g	3 days
E. coli water isolates	Modified m-TEC agar	Petri dish 50mm x 9mm	Ice/refrigieration	4°C	100 mL	24 – 48 hrs, then shipped to SAML on ice
Bacteroidales	Supor filters	7 oz. Whirl-Pak bag	GITC buffer	-20 or -80°C	100 mL	6 hours**, filters indefinitely

SSB: sterile sample bag, Whirl-Pak

PC HDPE: pre-cleaned high-density polyethylene

sterile PE: sterile poly-ethelyene container

**6 hours to deliver to laboratory. In the case that this 6-hour holding time is not met, the E. coli quantitative count will be flagged and not reported, though the Bacteroidales PCR will still be valid.

Storm Sampling Procedures

Storm event runoff water samples will be collected using refrigerated ISCO Avalanche samplers collecting from flow the stream channel. Water level in the stream, date, time, and collector's name will be recorded at the time the sample is retrieved from the sampler. Water samples will be collected based on flow volume, not time. Samplers will be triggered when flow level in the stream increases by 5% from ambient conditions within a 6 hour period. Samples will continue to be collected based on a flow-weighted average until samples are collected, the single sterile sample bottle is full, or stream depth returns to pre-event levels. The flow weighted average sampling trigger varies between the Attoyac Bayou and Big Iron Ore Creek due to their respective sizes and flow volumes. The sampler for the Attoyac Bayou pulls samples after each 100,000 ft^3 passes the sampler and the sampler on Big Iron Ore Creek is set to collect samples after each 15,000 ft^3 passes the sampler. After the first sample is collected until the completion of the running program, the Avalanche cools the refrigerated compartment to 1^oC +/- 1. One hour after the last sample of the program is taken, the Avalanche adjusts its control to maintain the samples at 3^oC +/- 1. These temperatures are both within the $<10^oC$ temperature requirement for non-regulatory storm samples. The 5 gal collection bottle will be removed from the sampler and transported to the laboratory on ice. A new (clean, sterilized sampling bottle) will be placed in the sampler in preparations for the next sampling event. From this point forward, samples will be treated identically to routine samples collected with the only exception being that once the sample bottle arrives at the lab, it will be thoroughly mixed and sub-samples will be drawn into sterile containers for sample analysis (see table B2.1 for listing of analyses). Field sampling procedures previously described will also be carried out for storm sampling events with the exception of stream flow readings. Flow data are obtained by retrieving it from the continuous flow record at the respective sampling site. This data collection process is described below.

Adherence to prescribed holding times as listed in Table B2.1 is achieved through automated notifications. When sampling is initiated, field staff is notified by phone that the sampling unit(s) has been activated. At that point, field staff begins planning to retrieve samples and deliver them to the laboratory within the prescribed holding time.

Stormflow data are submitted to TCEQ as "biased flow" samples and are therefore not used for regulatory (water body assessment) purposes and thus are not required to adhere to a 6 hour holding time.

Continuous Flow Monitoring

Each ISCO Avalanche is triggered to collect stormwater samples by a change in water depth as described above. Depth and water velocity are recorded using a SonTek Argonaut SW pulsed acoustic Doppler velocity meter. Using this data, flow volume is calculated using the FlowPack software supplied with the Doppler unit and the measured channel dimensions. These data are calculated on 15 minute intervals. This approach is known as the area velocity method and is described in the *ISCO Open Channel Flow Measurement Handbook* (Walkowiak 2006).

Failures in Sampling Methods Requirements and/or Deviations from Sample Design and Corrective Action

Examples of failures in sampling methods and/or deviations from sample design requirements include but are not limited to such things as sample container problems, sample site considerations, etc. Failures or deviations from the QAPP are documented on the field data reporting form and reported to the SFASU Project Leader. The SFASU Project Leader will determine if the deviation from the QAPP compromises the validity of the resulting data. The SFASU Project Leader, in consultation with the TSSWCB PM and QAO, will decide to accept or reject data associated with the sampling event, based on best professional judgment. The resolution of the situation will be reported to the TSSWCB in the quarterly progress report (QPR).

Fecal Sampling Method Requirements

Fecal samples will be obtained one of three ways: 1) collecting fecal samples from areas where animals were visually observed defecating by technician; i.e. deer or feral hogs at feeders; and 2) gut samples collected from animals recently killed by cars (within 24 hours) or 3) legally harvested by hunters who have agreed to work with the technician. Gut samples will be collected by using sterile loops inserted anally or by cutting into the intestine using a sterile scalpel.

LDC and SELECT Data Analysis
Not relevant.

RUAA
Field sampling activities for recreational use attainability tasks shall at least satisfy those questions found on the Field Data Sheets, Interview Forms, and Summary Sheets as specified by the TCEQ *Procedures for a Comprehensive RUAA and a Basic RUAA Survey* (TCEQ 2009). Versions of these forms for this project are found in Appendix H.

BST Analysis
All samples used in BST analysis will be collected and prepared by SFASU prior to shipment to SAML.

Section B3: Sample Handling and Custody Requirements

Land Use/Land Cover Update

Not relevant.

Surface Water Quality Monitoring

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 COC form is used to document sample handling during transfer from the field to the laboratory and inter-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. Sample information will be entered into ANRA's Laboratory Information Management System (LIMS) upon receipt of the samples. The LIMS will generate a unique sample identification number for the sample, which will be affixed to each container. A sample receipt log will be printed each day and maintained on file. A copy of a blank COC form used on this project is included as Appendix D.

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 (ANRA or SFASU SPWAL). 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.

Following sample preparation, plates containing *E. coli* cultures will be stored at 4°C in a refrigerator for a maximum of 24-48 hours before shipment to SAML. Following *Bacteroidales* sample preparation, filters will be stored at SFASU SPWAL in a -20°C manual defrost freezer or an ultra-low (-80°C) freezer until delivery to SAML is arranged. Cultured *E. coli* samples will be delivered, overnight, from SFASU SPWAL to SAML in a cooler box with appropriate refrigerant methods to maintain appropriate temperatures; *E. coli* isolates will be shipped on blue ice or freezer blocks and *Bacteroidales* samples will be shipped on dry ice. Any problem will be documented with a CAR.

Specific shipping and handling methods for *E. coli* and *Bacteroidales* are clearly outlined in Appendices E-4 and F.

Failures in Chain-of-Custody and Corrective Action

All failures associated with COC procedures are to be immediately reported to the TSSWCB PM. Failures 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 Project Leader and the TSSWCB PM/QAO will determine if the procedural violation may have compromised the validity of the resulting data. Any failure that potentially compromises 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 QPR. Copies of the CARs will be maintained by the appropriate Laboratory Supervisor(s), TWRI PM, and TSSWCB PM.

LDC and SELECT Data Analysis

Not relevant.

RUAA

Not relevant.

BST Analysis

The same sample handling and custody procedures followed under the ***Surface Water Quality Monitoring*** section above apply here.

Section B4: Analytical Methods

Land Use/Land Cover Update

Phase 1 Classification:

The CES is using a combination of satellite based image classification schemes and heads-up digitizing of NAIP 2004 (leaf-on) and 2009 (leaf-off) aerial imagery of the area to conduct the land cover inventory of the watersheds. NAIP quarter quads are rectified to the UTM coordinate system, NAD 83 and cast into a single predetermined UTM zone.

The spectral classes from each scene covering the watersheds are first labeled into the twelve LULC categories using whatever ground information was available, including aerial photos, topo maps and data from the NRCS. The land use classification scheme to be used is described in Section A6. Individual LULC classes will be identified and delineated in shapefile or ArcGIS grid format with a minimum mapping unit of 2 acres on screen. Ground truth sample polygons are then divided into two randomly selected groups, one for image labeling and the other for classification accuracy testing.

Phase 2 Classification:

ESRI ArcGIS software will be used to classify images in Phase 2. Classification will be done using the geographic extents of one scene. The product of the Phase 1 classification will be used as input to the supervised classification process. One category will be selected as the focus of a classification operation. Appropriate ground samples and ancillary polygons containing LULC data, located and labeled by CES personnel, will be matched with corresponding areas on the original satellite images and the image polygons will be classified using on-screen interpretive techniques to a total average accuracy of 80% or greater. The process will be repeated for each LULC category using field samples and other ancillary data.

As a point of comparison, USGS NLCD 2001 data is created with Landsat Thematic Mapper images. Each image is precision terrain-corrected using 3-arc-second DTED, and georegistered using ground control points. The resulting root mean square registration error is less than 1 pixel, or 30 meters.

A detailed account of data processing techniques will be documented in metadata according to the established standards. ESRI ArcCatalog software will be used to record the metadata for this project.

Surface Water Quality Monitoring

The analytical methods, associated matrices, and performing laboratories are listed in Table A7.1 of Section A7. Procedures for laboratory analysis will be conducted in accordance with the most recently published edition of *Standard Methods for the Examination of Water and Wastewater*, the latest version of the *SWQM Procedures, Volume 1: Physical Methods for*

Water, Sediment, and Tissue, 40 CFR 136, or other reliable procedures acceptable to the Executive Director to ensure consistency with the Clean Rivers Program and its data collection and analysis requirements.

Laboratories collecting and analyzing data under this QAPP are compliant with the NELAC standards where required. Copies of laboratory QMs, QAPP and documented methods guidance are available for review by the TSSWCB.

Standards Traceability
All standards used in the field and laboratory are traceable to certified reference materials. Standards preparation is fully documented and maintained in a standards log book. Each documentation includes information concerning the standard identification, starting materials, including concentration, amount used and lot number; date prepared, expiration date and preparer's initials/signature. The reagent bottle is labeled in a way that will trace the reagent back to preparation.

Analytical Method Deficiencies and Corrective Actions
Deficiencies 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 SFASU SPWAL / ANRA Laboratory Supervisor, who will make the determination and notify the SFASU SPWAL / ANRA QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB. The nature and disposition of the problem is reported on the data report which is sent to the SFASU SPWAL / ANRA PM. This information will be included in the CAR and submitted with the Progress Report which is sent to the TSSWCB PM.

The definition of and process for handling deficiencies and corrective action are defined in Section C1. The TCEQ has determined that analyses associated with the qualifier codes "holding time exceedance", "sample received unpreserved", "estimated value", etc... may have unacceptable measurement uncertainty associated with them. This will immediately disqualify analyses from submittal to SWQMIS. Therefore, data with these types of problems should not be reported. Additionally, any data collected or analyzed by means other than those stated in the QAPP, or data suspect for any reason should not be submitted for loading and storage in SWQMIS.

LDC and SELECT Data Analysis
Not relevant.

RUAA
Not relevant.

BST Analysis

The analytical methods utilized in BST analysis and sample preparation are listed in Table B4.1 and described in detail in Appendix E.

E. coli in water samples will be quantified and isolated by SFASU SPWAL personnel using modified mTEC agar, EPA Method 1603 (USEPA 2006). The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with MUG observed by ultraviolet light fluorescence.

E. coli colonies from the modified mTEC medium will be picked by SAML and streaked for purity on nutrient agar with MUG to confirm glucuronidase activity and culture purity SAML. Cultures of selected isolates will be archived using glycerol freezing medium (-80^{o}C). Inoculated plates will be incubated at 35±0.5^{o}C for 2 hours to resuscitate stressed bacteria, then incubated at 44.5±0.2^{o}C for approximately 20 to 24 hours. *E. coli* isolates obtained from ambient water samples from across the study area will be characterized using ERIC-PCR and RiboPrinting using EP AREC SOPs. DNA patterns of those isolates will be compared to the Texas Known Source Library of *E. coli* isolates from known animal and human sources collected throughout Texas. Water isolates will be identified to cattle, other livestock, avian and non-avian wildlife, domestic sewage, and pet sources (six-way split), as well as a broader three-way split of livestock, domestic sewage and wildlife.

As outlined in Appendix E, 100 ml water samples will be collected and filtered for analysis of *Bacteroidales*. *Bacteroidales* DNA will be extracted from the filters and analyzed using PCR using EP AREC SOPs.

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.

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, QC 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 SAML Director, who will make the determination in coordination with the TWRI PM/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 PM/QAO will include this information in the CAR and submit with the QPR which is sent to the TSSWCB PM.

Table B4.1. Laboratory Analytical Methods

Parameter	Method	Equipment Used
Laboratory Parameters		
E. coli	EPA 1603	Filtration apparatus, incubator
E. coli	SM 9223-B	
E. coli RiboPrint fingerprint	EP AREC SOP	RiboPrinter
E. coli ERIC-PCR fingerprint	EP AREC SOP	PCR thermal cycler, gel electrophoresis apparatus
Bacteriodales PCR	EP AREC SOP	PCR thermal cycler, gel electrophoresis apparatus
TSS	SM 2540 D	
Ammonia-N, total	SM 4500NH3-C or D	
Nitrate/Nitrite-N	SM 4500NO3-E	
Dissolved Ortho-P	SM 4500-PE	
Total P	SM 4500-PE	
Field Parameters		
pH	EPA 150.1 & TCEQ SOP, V1	YSI Multi-probe
DO	SM 4500-O G & TCEQ SOP, V1	YSI Multi-probe
Specific Conductance	EPA 120.1 & TCEQ SOP, V1	YSI Multi-probe
Temperature	SM 2550 B & TCEQ SOP, V1	YSI Multi-probe
Days since last significant rainfall	TCEQ SOP, V1	Field Observation
Flow	TCEQ SOP, V1	Electronic flow meter
Flow (automated sampling)	ISCO Open Channel Flow	Doppler flow meter
Total water depth	TCEQ SOP, V1	Meter Stick
Flow measurement method	TCEQ SOP, V1	Electronic flow meter
Flow severity	TCEQ SOP, V1	Field Observation
Present weather	TCEQ SOP, V1	Field Observation

EPA = Methods for Chemical Analysis of Water and Wastes, March 1983
SM = Standard Methods for Examination of Water and Wastewater, 21st edition
TCEQ SOP, V1 = TCEQ's Surface Water Quality Monitoring Procedures, Volume 1

Section B5: Quality Control Requirements

Land Use/Land Cover Update

Assessing the accuracy of land cover mapping products is an elusive and challenging problem that calls for continuing research and development within GIS and remote sensing technology. The criteria for accuracy assessment reflect the need to balance the requirements for rigor and defensibility with practical limitations of cost and time. The assessment methods must be scientifically sound and economically feasible.

The basic unit of the land cover mapping process is a polygon of 2 acres that represents a land use / land cover class with a relatively homogenous composition; waterbodies will be accounted for at a smaller scale of ½ acre. An accuracy assessment will be conducted by selecting a sample of locations (e.g., centroids of mapped polygons) from the final version of the land cover map and determining the true land cover classification at these locations. These data are frequently called the reference data set. Properly executing an accuracy assessment involves knowing the nature of the created map, identifying the field methods for obtaining the reference data, designing a sound method for selecting reference data, actually collecting the data, conducting statistical analyses, and reporting the results.

This project has a goal of mapping land cover with 80% accuracy. We will attempt to measure thematic accuracy as a percentage of the land cover map classified correctly overall and by cover type with a standard error no greater than 8%.

Summary of steps and standards used in Accuracy Assessment:

1. Produce a final land cover map, classification, and description of land cover classes that will be assessed.
2. Identify the methods for obtaining reference data.
3. Design a sampling protocol that meets the desired statistical precision.
4. Collect the reference data, test their reliability, and archive the database.
5. Compare the reference data to the map, conduct analyses, and report the results.

Step 1: A final version of a land cover map will be produced as described in section B4. We anticipate having at least 12 cover classes that can be delineated on the satellite imagery. Because classification will be done in phases, one scene at a time, it will not be necessary to wait until the mapping is completed for all watersheds to begin accuracy assessment. Knowledge of the characteristics of the map to be assessed is important in determining the sampling frame (number, size, and classification of polygons). The methodology used to collect the reference data will match the classification system of the cover map.

Step 2: We plan to use field collected data as the primary source of reference data to assess the quality of the final cover map. Ground-truthing involves physically visiting the site in question to determine its true land cover type and will require substantial cooperator support and coordination. CES will develop a field sampling plan that will guarantee consistency

between reference data and the needs of the assessment project and future remapping, (i. e., the method of collecting the field data will enable the land cover to be identified at the same level of detail as the land cover map). QC will be achieved by assuring that the GPS receiver performance criteria under section A.5 above are met at all times. Statistical checks will be performed on the data during the post-processing phase and the data will be compared to known map coordinates and features using USGS topographic maps and other appropriate map sources of known quality.

The design of the assessment study will be stratified by, and only by, land cover types present in the final land cover map. The protocol for selecting field sampling sites will be based on the final number of land cover classes, the number of polygons within each class, and the number of samples needed to accomplish statistical precision.

With a minimum mapping unit of 2 acres (½ acre for waterbodies), we anticipate that the occurrence of other unmapped cover types (inclusions) within a polygon will cause few problems in collecting field data. Nevertheless, CES will develop field protocols to ensure that each mapped cover type can be correctly identified in the field. The characteristics of land cover types that may affect these protocols are: polygon sizes (small, medium, large), polygon shapes (linear or non-linear), and heterogeneity of the land cover (degree of patchiness and size of inclusion patches).

An individual measurement will result in a decision as to whether or not the field reference point agrees with the land cover map's label of that polygon. Accuracy is the statistical reduction of many samples into a statement of percent agreement.

Step 3: Sampling units are defined here as all areas within the project area geographically contiguous and of homogenous primary attribute, that is, vector polygons or contiguous raster clusters of the same primary land cover type code. Land cover maps are based on algorithmic clustering of TM pixels with the resultant categories being spectrally similar. Therefore, pixels are probably not independent of each other. Although polygon boundaries are not precise, they are believed to represent real patterns on the ground and the polygon is the defined feature that should be assessed. Therefore, the sampling unit is defined as a mapped polygon. The sample frame is the list of all polygons that comprise the final land cover map.

The sampling protocol for accuracy assessment will be designed to meet the statistical precision needed to accomplish the stated objectives for accuracy and standard error. Field sites will be selected through a stratified, two-stage probability sample. Accuracy assessment field data will be recorded on forms and returned to the CES for analysis (see Appendix B). Probability sampling, as opposed to purposive selection of "representative" elements or haphazard selection of convenient elements, is now a standard scientific tool since it guards against selection biases and it leads to objective statistical inferences. Stratification will ensure good geographic spread of the sample across the state and will provide a representative sample of alliances.

Two stages of sampling will be employed. In the first stage, large tracts of land (e.g. counties, Landsat scenes, or some other convenient unit) will be selected in a stratified sample. In the second stage, sampling points within the large tracts will be selected. The reason for sampling in two stages, as opposed to sampling sites directly, is that direct sampling of sites would lead to a widely-scattered sample with high logistical costs.

Because cost of collecting field data could be limiting, consideration will be given to stratifying according to the relative cost or effort required to measure the sampling site.

Step 4: GIS methods will be used to select sampling units from the sampling frame which consists of all the polygons in a vector map.

Field surveys will use methods similar to those used to collect data for classification purposes (Appendix B). However, reference data will be collected by 2 or 3 well-trained field observers who have no knowledge of the primary attribute given by the land cover map for the sampling unit. This will involve providing each observer with coordinates and a map showing the polygon to be sampled but without the associated land cover type label. The field maps will typically have base information such as roads, streams, and locational grids such as UTM coordinates.

Observers will be trained and field tested in the typical techniques used for land use inventories. They will also be given training in the classification scheme employed in the land cover mapping process. They will be provided written guidelines and other materials to assure that consistent, repeatable results are obtained (Appendix B).

The field data for each sampling unit will be assigned a pointer that identifies its location on the land cover map. Reference data will be compiled as a GIS coverage containing both the locations of samples and their attributes. Metadata will include a description of the method used by the analyst to determine agreement between the map and reference data and a measure of observer reliability in order to replicate the published analysis. Field forms will be archived and GIS data managed in accordance with procedures outlined in this document.

Step 5: Measurements from field sampling units will be compared with labeled polygons on the land cover map. As a first step in statistical analysis, agreements, or lack thereof, will be tabulated in a matrix whose rows represent mapped categories and columns represent observed cover types. The resulting error matrix is a contingency table which represents the probabilities of every possible correct or incorrect classification.

Statistical analyses of the measurements from the assessment sample need to recognize that the data arise from a complex sample. It is not valid to analyze these data as if they are independent and identically distributed. Analyzing data from a stratified two-stage sample as if they were independent and identically distributed will typically lead to confidence intervals which are unrealistically narrow and hypothesis tests which reject too easily. That is, the

precision of the analysis is overstated. Proper methods for dealing with data from stratified two-stage samples will be employed in this study.

Limitations and Constraints: In planning accuracy assessments, three general constraints (technology, logistics, and cost) must be considered because of the limitations they place on our ability to obtain ideal data sets.

Technological constraints: This category of constraints includes measurement errors relating to acquiring field observations. Error in determining the true location of the sampling unit in the field should not be a major problem in Texas because the terrain is moderate and bisected by an elaborate system of roads and highways. Sampling units will be outlined in advance on topographic maps, county road maps, and aerial photos (if available) and provided to field observers. Also, field observers will usually be able to survey entire sampling units, thereby reducing error caused by inadequate integration of all attributes of a unit.

Logistical constraints: Most sampling units will be located in close proximity of a road and can be visited without great expense. Few locations will be inaccessible due to dangerous terrain. If sampling measurements cannot be made at a site due to inaccessibility, then these sites will be dropped from the sampling scheme and replaced with more accessible ones.

Surface Water Quality Monitoring

Sampling Quality Control Requirements and Acceptability Criteria

The minimum Field QC requirements are outlined in the *TCEQ Surface Water Quality Monitoring Procedures.* Specific requirements are outlined below. Field QC sample results are submitted with the laboratory data report (see Section A9). ANRA and SFASU SPWAL will utilize these QC requirements as required for each respective analysis conducted.

Field Split

A field split is a single sample subdivided by field staff immediately following collection and submitted to the laboratory as two separately identified samples according to procedures specified in the *SWQM Procedures*. Split samples are preserved, handled, shipped, and analyzed identically and are used to assess variability in all of these processes. Field splits apply to conventional samples only and are collected on a 10% basis. If less than ten samples are collected in a month, one set of field splits will be collected per month. The precision of field split results is calculated by relative percent difference (RPD) using the following equation:

$RPD = |(X_1 - X_2)/\{(X_1+X_2)/2\} * 100|$ (for nutrient parameters)

$RPD = (X1-X2)/((X1+X2)/2)$ (for *E. coli* analysis using EPA 1603)

A 30% RPD criteria will be used to screen field split results as a possible indicator of excessive variability in the sample handling and analytical system. If it is determined that

elevated quantities of analyte (i.e., > 5 times the LOQ) were measured and analytical variability can be eliminated as a factor, than variability in field split results will primarily be used as a trigger for discussion with field staff to ensure samples are being handled in the field correctly. Some individual sample results may be invalidated based on the examination of all extenuating information. The information derived from field splits is generally considered to be event specific and would not normally be used to determine the validity of an entire batch; however, some batches of samples may be invalidated depending on the situation. Professional judgment during data validation will be relied upon to interpret the results and take appropriate action. The qualification (i.e., invalidation) of data will be documented on the data summary. Deficiencies will be addressed as specified in this section under Quality Control or Acceptability Requirements Deficiencies and Corrective Actions.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

Batch

A batch is defined as environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of up to 20 environmental samples of the same NELAC-defined matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 25 hours. An **analytical batch** is composed of prepared environmental samples (extract, digestates or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various environmental matrices and can exceed 20 samples.

Method Specific QC requirements

QC samples, other than those specified later this section, are run (e.g., sample duplicates, surrogates, internal standards, continuing calibration samples, interference check samples, positive control, negative control, and media blank) as specified in the methods. The requirements for these samples, their acceptance criteria or instructions for establishing criteria, and corrective actions are method-specific.

Detailed laboratory QC requirements and corrective action procedures are contained within the individual laboratory QMs. The minimum requirements that all participants abide by are stated below.

LOQ Check Standard ***ANRA LAB ONLY***

An LOQ check standard 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 at the lower limits of analysis. The LOQ check standard is spiked into the sample matrix at a level less than or near the LOQ for each analyte for each analytical batch of samples run.

The LOQ check standard is carried through the complete preparation and analytical process. LOQ Check Standards are run at a rate of one per analytical batch. The percent recovery of the LOQ check standard is calculated using the following equation in which %R is percent recovery, SR is the sample result, and SA is the reference concentration for the check standard:

%R = SR/SA * 100

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

Laboratory Control Sample (LCS) ***ANRA LAB ONLY***

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 mid-point of the calibration for each analyte. In cases of test methods with very long lists of analytes, LCSs are prepared with all the target analytes and not just a representative number, except in cases of organic analytes with multi-peak responses.

The LCS is carried through the complete preparation and analytical process. LCSs are run at a rate of one per preparation batch. 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

A laboratory duplicate is prepared by taking aliquots of a sample from the same container under laboratory conditions and processed and analyzed independently. A laboratory control sample duplicate (LCSD) is prepared in the laboratory by splitting aliquots of an LCS. Both samples are carried through the entire preparation and analytical process. LCSDs are used to assess precision and are performed at a rate of one per preparation batch.

For most parameters, precision is calculated by the relative percent difference (RPD) of LCS 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:

$RPD = |(X_1 - X_2)/\{(X_1+X_2)/2\} * 100|$ (for nutrient parameters)
$RPD= (X1-X2)/((X1+X2)/2)$ (for *E. coli* analysis using EPA 1603)

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 lab. 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. 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 organisms/100mL.

Matrix spike (MS)
Matrix spikes are prepared by adding a known mass of target analyte to a specified amount of matrix sample for which an independent estimate of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency.

Percent recovery of the known concentration of added analyte is used to assess accuracy of the analytical process. The spiking occurs prior to sample preparation and analysis. Spiked samples are routinely prepared and analyzed at a rate of 10% of samples processed, or one per preparation batch whichever is greater. The information from these controls is sample/matrix specific and is not used to determine the validity of the entire batch. To the extent possible, matrix spikes prepared and analyzed over the course of the project should be performed on samples from different sites. The MS is spiked at a level less than or equal to the midpoint of the calibration or analysis range for each analyte. Percent recovery (%R) is defined as 100 times the observed concentration, minus the sample concentration, divided by the true concentration of the spike.

The results from matrix spikes are primarily designed to assess the validity of analytical results in a given matrix and are expressed as percent recovery (%R). The laboratory shall document the calculation for %R. The percent recovery of the matrix spike is calculated using the following equation in which %R is percent recovery, SSR is the observed spiked sample concentration, SR is the sample result, and SA is the reference concentration of the spike added:

$\%R = (SSR - SR)/SA * 100$

The results are compared to the acceptance criteria as published in the mandated test method. Where there are no established criteria, the laboratory shall determine the internal criteria and document the method used to establish the limits. The laboratory has established limits for

matrix spike recovery of 80-120% unless more stringent limits are mandated by the method. For matrix spike results outside established criteria, corrective action shall be documented or the data reported with appropriate data qualifying codes.

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 preparation batch. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the LOQ. For very high-level analyses, the blank value should be less than 5% of the lowest value of the batch, or corrective action will be implemented. 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.

The method blank shall be analyzed at a minimum of one per preparation batch. In those instances for which no separate preparation method is used (example: volatiles in water) the batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.

Positive Controls for *E. coli*

SFASU SPWAL will maintain live *E. coli* in tryptic soy broth and kept refrigerated until needed. Each time a set of samples is run a positive control will be performed in the lab using the same media and 1 ml of live *E. coli* which will be added to 99 ml of sterile distilled water that will be run through the filter funnel system and the filter placed on the media. This control should always be positive for *E. coli* after recommended incubation time.

Quality Control or Acceptability Requirements Deficiencies and Corrective Actions

Sampling QC excursions are evaluated by the SFASU SPWAL manager in consultation with the SFASU SPWAL QAO and the ANRA PM and QAO. In that differences in sample results are used to assess the entire sampling process, including environmental variability, the arbitrary rejection of results based on pre-determined limits is not practical. Therefore, the professional judgment of the SFASU SPWAL manager, SFASU SPWAL, ANRA PM and QAO will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility. Field blank values exceeding the acceptability criteria may automatically invalidate the sample, especially in cases where high blank values may be indicative of contamination which may be causal in putting a value above the standard.

Notations of field split excursions and blank contamination are noted in the quarterly report and the final QC Report.

Laboratory measurement quality control failures are evaluated by the laboratory staff. The disposition of such failures and the nature and disposition of the problem is reported to the ANRA Laboratory QAO. The Laboratory QAO will discuss with the ANRA PM. If applicable, the ANRA PM will discuss failures with pertinent project PMs and QAOs. The TWRI PM and QAO will include this information in the CAR and submit with the Progress Report which is sent to the TSSWCB PM.

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

LDC and SELECT Data Analysis

Not relevant.

RUAA

Not relevant.

BST Analysis

Table A7.1 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the SCSC Project Leader to verify that the data are representative. The SCSC 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 QA of field sampling methods will be conducted by the TSSWCB QAO or their designee.

Laboratory Blanks

For *Bacteroidales* PCR, a laboratory blank will be analyzed with each batch of samples to ensure no cross-contamination occurs during sample processing. In addition, negative controls will be analyzed for each batch of PCR samples.

Positive Control

Positive controls (a well-characterized *E. coli* strain or microbial community DNA from known fecal sources) will be analyzed by SAML for each batch of *E. coli* ERIC-PCR and RiboPrinting, and *Bacteroidales* 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 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 cfu/100 ml.

Failures in Quality Control and Corrective Action

Notations of blank contamination will be noted in QPRs 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 discussed with pertinent project PMs and QAOs. The TWRI PM and QAO will include this information in the CAR and submit with the Progress Report which is sent to the TSSWCB PM.

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

Land Use/Land Cover Update

Equipment testing will be accomplished by the GPS Operator prior to, during and after field use. Built-in equipment diagnostics and functionality checks will be utilized in accordance with the operation manuals. Results will be reported in pre-survey, field and post-processing logs. Issues will be documented with the GPS Coordinator or equipment owner.

Surface Water Quality Monitoring

All sampling equipment testing and maintenance requirements are detailed in the *TCEQ Surface Water Quality Monitoring Procedures (Volume 1)* October 2008. Sampling equipment is inspected and tested upon receipt and is assured appropriate for use. Equipment records are kept on all field equipment and a supply of critical spare parts is maintained.

All laboratory tools, gauges, instrument, and equipment testing and maintenance requirements are contained within laboratory QM(s).

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 QPR. The CARs will be maintained by the SFASU Project Leader, TWRI QAO and the TSSWCB PM.

LDC and SELECT Data Analysis

Not relevant.

RUAA

Same procedure as for field monitoring equipment described in the ***Surface Water Quality Monitoring*** section described above.

BST Analysis

To minimize downtime of all measurement systems, spare parts for laboratory equipment will be kept in the laboratory, and all laboratory equipment must be maintained in a working condition. All laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendations in *Standard Methods for the Examination of Water and Wastewater, 21st Edition*. Maintenance and inspection logs will be kept on each piece of laboratory equipment.

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 QPR. The CARs will be maintained by the SCSC Project Leader, TWRI PM and QAO as well asthe TSSWCB PM.

Table B6.1. Equipment Inspection and Maintenance Requirements

Equipment	Relevant Testing, Inpection & Maintenance Requirements
Thermometers PCR Thermal cycler RiboPrinter Water deionization units Media dispensing apparatus Autoclaves Refrigerator Ultra Low Freezer Membrane filter equipment Ultraviolet sterilization lamps Biological safety cabinet Incubators Glassware and plastic ware Utensils and containers Dilution water bottles SonTek Argonaut SW 9000 ISCO Avalanche Sampler	Per Manufacturer and Annual Preventative Maintenance Guidance for Each Specific Instrument or Equipment Item

Section B7: Instrument Calibration and Frequency

Land Use/Land Cover Update

GPS receivers cannot be calibrated. However, a number of settings can be changed (maximum PDOP, signal-to-noise ratio, filter coefficient, etc.) which will affect operation of the unit. In general, manufacturer default settings will be employed for optimum data accuracy.

Surface Water Quality Monitoring

Field equipment calibration requirements are contained in the *TCEQ Surface Water Quality Monitoring Procedures*. Post-calibration error limits and the disposition resulting from error are adhered to. Data not meeting post-error limit requirements invalidate associated data collected subsequent to the pre-calibration and are not submitted to the TCEQ.

The SonTek Argonaut-SW automated flow monitoring equipment does not require instrument calibration.

Detailed laboratory calibrations are contained within the QM(s).

LDC and SELECT Data Analysis

Not relevant.

RUAA

Same procedure as for field monitoring equipment described in the ***Surface Water Quality Monitoring*** section described above.

BST Analysis

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 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 a laboratory audit.

All instruments or devices used in obtaining environmental data will be used according to appropriate laboratory practices. Written copies of EP AREC SOPs are available for review upon request and are attached as Appendix E in this QAPP.

Standards used for instrument or method calibrations shall be of known purity and be NIST traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society 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 QPR. The CARs will be maintained by the SCSC Project Leader and the TSSWCB PM.

Table B7.1. Instrument Calibration Requirements

Equipment	Relevant Testing, Inpection & Maintenance Requirements
PCR Thermal cycler	Per Product Owner's Manual
RiboPrinter	

Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

Land Use/Land Cover Update

The primary consumables for GPS operations are batteries. During the equipment testing, inspection and maintenance periods, batteries will be examined by the GPS Operator for functionality, charge and compatibility with manufacturer's specifications. Fully charged, backup batteries will be taken to the field for use when recharging is not an option.

Supplies used by CES will be inspected upon receipt by CES for visible signs of damage. All data will be backed up on removable storage media so that failure of primary storage media will not result in data loss. Supplies will be purchased from reputable vendors to ensure quality.

Surface Water Quality Monitoring

SFA personnel will coordinate with ANRA and use identical sampling containers and apparatus as ANRA does through their CRP monitoring. These include pre-cleaned sterile sample bottles. All other miscellaneous consumable supplies such as batteries and office supplies are purchased where needed.

All consumable laboratory supplies are purchased from reputable scientific supply dealers.

LDC and SELECT Data Analysis

Not relevant.

RUAA

Not relevant.

BST Analysis

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 QC 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.

Section B9: Data Acquisition Requirements (Non-direct Measurements)

Land Use/Land Cover Update

The display of GPS ground points will be accomplished by overlaying the collected points on map features of comparable quality. This provides a road network, topographic features and other map elements that can place the collected points in the context of real-world features. This is an additional quality check, since large deviations from expected locations would cause the data and processing methods to be rechecked. Standards map products of known quality will be used.

NAIP 2009 satellite imagery will be the primary data source for constructing base maps of LULC. Winter coverage from 2009 has been obtained and will be cross referenced with NLCD 2001 imagery and NAIP 2004 leaf-on infrared imagery. Ancillary information will be drawn from other imagery where applicable.

The NAIP data are downloaded electronically from the Texas Natural Resource Information System. The images have been pre-processed to correct missing information and other problems inherent in satellite-gathered imagery. The images obtained were also geo-referenced to real-world coordinates and clustered into 240 spectrally distinct classes prior to receipt.

NAIP aerial photos of the area will be used to provide two main products: 1 meter GSD ortho imagery rectified to a horizontal accuracy of within +/- 3 meters of reference DOQQs from the NDOP (2004 imagery); and, 2 meter GSD ortho imagery rectified to within +/- 20 meters of reference DOQQs (2005 imagery). The tiling format of NAIP imagery is based on a 3.75' x 3.75' quarter quadrangle with a 360 meter buffer on all four sides. NAIP quarter quads are rectified to the UTM coordinate system, NAD 83 and cast into a single predetermined UTM zone.

Because most historical data is of known and acceptable quality and were collected and analyzed in a manner comparable and consistent with needs for this project, no limitations will be placed on their use, except where known deviations have occurred.

GIS Inventory

The GIS inventory describe in section A6 utilizes secondary data readily available from a variety of electronic outlets. These data are simply compiled into a working GIS and utilized for map making/informational purposes. The GIS inventory will include the most recent information available on land use, elevation, soils, stream networks, reservoirs, roads, public park lands, municipalities, rural census block data, 911 address location data and satellite imagery or aerial photography. Locations of SWQM stations, USGS gauges, public access points to the waterbodies, floodwater-retarding structures, wetlands, TPDES permittees (including WWTFs, CAFOs and MS4s), oilfield activities, poultry farms, and subdivisions will also be included if applicable. Sites permitted for land application of sewage sludge and septage should be included.

Because most historical data is of known and acceptable quality and were collected and analyzed in a manner comparable and consistent with needs for this project, no limitations will be placed on their use, except where known deviations have occurred.

Surface Water Quality Monitoring

Water quality data available in TCEQ's SWQMIS will be used as a historical reference for instream water quality and conditions. These data will support the development of LDCs and the waterbody assessment. This is the only water quality data collected outside this project that will be utilized.

LDC and SELECT Data Analysis

Water quality data collected by the ANRA through their CRP monitoring, specifically *E. coli*, ammonia and flow, will be used along with data collected through this project to conduct the SELECT (*E. coli* only) and LDC (*E. coli* and ammonia) analyses. The ANRA is a partner in the Clean Rivers Program for the state of Texas. As such, they collect data on a regular basis for routine water quality assessment as part of the state's mandate for CWA §305(b) – Water Quality Inventory Report. These data also are used by Texas for consideration of water bodies to be added to their list of impaired water body segments, as described in CWA §303(d). Additional data obtained from the Texas Commission Environmental Quality (TCEQ) are from the SWQMIS database.

Data collected under this project will also be used to develop SELECT and LDC analyses. These data will be collected in accordance with the approved QAPP for the project and will be collected by SFA as well. Data that may be used from this project include water quality, rainfall and streamflow information.

All data used in the modeling procedures for this project are collected in accordance with approved quality assurance measures under the state's Clean Rivers Program, TCEQ, Texas Water Development Board, USDA, National Weather Service, or USGS.

GIS data to be used are 2004 and 2009 National Agricultural Imagery Program aerial photos, SSURGO and Computer Based Mapping System soils, 2001 USGS (NLCD landuse, National Hydrography Dataset, Census data (2010), 911 address locations as maintained by regional councils of government, Agricultural Census data from USDA-National Agriculture Statistics Service (2007), and the USGS 30-meter resolution digital elevation model. Depending on the availability of the GIS layers from different data sources, efforts will be made to update the spatial data to the most recent year.

Because most historical data is of known and acceptable quality and were collected and analyzed in a manner comparable and consistent with needs for this project, no limitations will be placed on their use, except where known deviations have occurred.

RUAA

In order to obtain information on existing and historical uses and stream characteristics, CES and SFASU will conduct interviews of 1) users present during the field surveys, 2) streamside landowners along the field survey transects, 3) local residents, and 4) commercial providers of outdoor recreation goods and services. Survey instrument shall include at least those questions found on the Interview Form from the TCEQ *Procedures for a Comprehensive RUAA and a Basic RUAA Survey* (TCEQ 2009).

Historical documents will also be considered in this assessment. Information such as newspaper articles, published reports, online resources and other information found will be considered. As these are not analytical data, no standards or limitations will be set on these sources. As a result, all data found regarding past uses of the Attoyac Bayou will be considered.

BST Analysis

Data analyzed using BST analysis methods for this project will consist solely of data produced during the course of this study and will adhere to the guidance set forth in this QAPP.

Section B10: Data Management

Land Use/Land Cover Update

Field Collection

Field staff will visit each watershed to collect ground control points for at least ten locations per land use type using Garmin GPS 72 GPS Receivers with an accuracy of 20 ft. Site identification, date, time, personnel, and conditions at the site are noted in the field notebook.

All field observations 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, Vista or 7 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 r/w CD's weekly 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 one year, and then stored with the paper files for the remaining 4 years.

Spatial Data

NAIP is downloaded electronically and it is copied to the hard drive of a workstation. Data forms with field information arrive via hand-delivery or the US mail and are stored in raw form in the lab. Data from the forms are digitized and stored on the hard drive of a computer in the lab. Backup copies of all digital data are made to removable media. All data forms are checked prior to digitizing for accuracy and then after digitizing to assure correspondence to the original form. All necessary data from ancillary sources are digitized or copied to the hard drive of a computer in CES and then backup copies are made of the digital data. Where ancillary data have been digitized, CES checks that the original data correspond correctly to the digitized data.

A combination of IBM compatible microcomputers with a Windows XP, Vista or 7 Operating System and workstations will be used to process the data. An effort was made to purchase machines with the most memory, largest hard drives and fastest processing speeds that were available at the time. Additional hard drive space and random access memory will be purchased as project needs require. A suite of software will be used to process the data. All software packages are industry standard and represent the best application available for each processing function.

All GIS and LULC data will be backed up on r/w CD's weekly and stored in separate area away from the computer. At least 10% of all data manually entered in the database will be reviewed for accuracy by CES to ensure that there are no transcription errors. Hard copies of data will be printed and housed in CES for a period of five years.

This same protocol also applies for storage of spatial data compiled in the GIS inventory.

Data Validation

Following LULC classification and delineation, LULC data will be validated and verified with field sampling ground control points to accuracy of 80% or greater. Any LULC that does not meet this will be re-classified until an accuracy of 80% is achieved. No LULC that does not achieve 80% accuracy will be submitted to the TSSWCB.

Metadata Preparation

Metadata preparation will be accomplished by the GPS Operator upon conclusion of the data processing phase using the *Geospatial Metadata Technical Specification, Version 1.0* (EPA 2007c).

Data Dissemination

As classification of each watershed is completed, the PM will provide a copy of the shapefile or ArcGIS grid format of the LULC via recordable CD media to the TWRI and TSSWCB PM.

Surface Water Quality Monitoring

It is imperative that data and associated applications be maintained and managed in a manner consistent with the development and use of the data; in this case, data will be maintained so that they are consistent with CRP requirements. For scientifically valid results, the data, program applications, and reports must be handled in an orderly and consistent manner. Documented quality assurance and quality control checks/procedures are applied to all received data sets, individual data points and data manipulation programs.

Data will be incorporated into the ANRA database and subject to varying levels of review. The QA/QC checks evaluate each data set as a whole, and the validity of individual data points. Each data set to be processed into the database is evaluated for any problems that might impose a limitation on the use of the data. This check is performed prior to processing/importing to the database. The following information is considered:

a. Credibility of data source
b. Acceptable QA/QC procedures
c. Intended use of the data
d. Frequency of data collection/impact of missed sampling events
e. Sample size
f. Sample collection and preservation methods
g. Field and laboratory test procedures
h. General documentation

Upon passing the evaluation of a data set's limitations, the data are incorporated into the ANRA Database. Initially data are entered, either manually or electronically, into a set of working directory files that are consistent with the ANRA Database file structures. In the

event that a deviation is found in the data set, the corresponding data points will be coded with a "D" in the remarks section of the Results Table. The remark "D" code refers to the SWQMIS data qualifiers, which means 'did not pass all QC criteria. Any deviation found in the data set will be conveyed to the TWRI PM by ANRA. Disqualified data will be removed from the dataset and will not be submitted to the TSSWCB for inclusion in SWQMIS. The reason for the data removal will be listed on the data summary.

Electronic data input procedures vary according to the source and format of the data. Manual data input will be made to appropriately structured MS Access tables. Standardized procedures are followed to ensure proper data entry.

After the data/data sets have been input/converted into an appropriate working directory database, the individual data points will be evaluated to determine their reasonableness. Data values that are considered outliers will be discarded or coded prior to entry into the records directory. The criteria for determination of outliers will be based on individual data sets being processed for entry into the TCEQ's SWQMIS database. Once the data set is complete, any individual points falling outside the most recent Max/Min range as defined by the TCEQ SWQM Parameters Table will be considered outliers. If an outlier does occur, then it will be noted in the remark section of the database and verified against the original data report, and if necessary, verified by the laboratory. After verification, outliers will either be assigned the appropriate remark code or documented as verified with a 1 in the verify_flg section of the results table.

After the final QA checks are performed by ANRA, data are submitted to the TSSWCB PM. Data are then transferred from the TSSWCB PM to the TCEQ CRP Data Manager, who then loads the data into SWQMIS.

Only data collected under this project and its QAPP will be transferred. The tag series transferred is documented on the Data Summary (QAPP Appendix F) that is submitted to the TCEQ upon the completion of the data transfer. All QA data sets associated with the data transfer will be submitted in the form of a QA Table. The files are transferred as pipe delimited text file format as described in the *Surface Water Quality Monitoring Data Management Reference Guide* (TCEQ 2010) to the TSSWCB PM. After data have been transferred, reviewed, and loaded into the TCEQ Database, a link will provided to the TCEQ's Surface Water Quality Web Reporting Tool at http://www8.tceq.state.tx.us.SwqmisWeb/public/index.faces for public access.

Data Dictionary - Terminology and field descriptions are included in the *SWQM Data Management Reference Guide*, (TCEQ 2010). For the purposes of verifying which entity codes are included in this QAPP, the following will be used when submitting data under this QAPP:

Name of Monitoring Entity:	Stephen F. Austin State University WET Center
Tag Prefix:	TX
Submitting Entity:	Texas State Soil and Water Conservation Board (TX)

Collecting Entity: Stephen F. Austin State University WET Center (SF?)

Data Errors and Loss

To prevent loss of data and minimize errors, all data generated under this QAPP are verified against the appropriate quality assurance checks as defined in the QAPP, including but not limited to chain of custody procedures, field sampling documentation, laboratory analysis results, and quality control data. The data are also verified by comparing 10% of the data in the database to hard copy reports as a check against transcription errors.

Backup/Disaster Recovery Requirements

All data associated with ANRA's database and network files are completely backed-up daily. See record keeping and data storage section below for more details. The IBM Server PC is protected by an Internet Office UPS with battery backup and surge protection to safely work through blackouts and save open network files.

Should the computer system or software fail, ANRA will request the assistance of a Computer/Network Specialist to evaluate the probable cause of the failure, methods to prevent reoccurrence of the problem, and guide recovery of the system. The archived tape backups will allow for complete recovery of the hard disk drive contents.

Record Keeping and Data Storage

A three ring binder will be used as a data set log to track all hard copy data sets associated with the ANRA Database. The database management log will also record the structure of tables, data modifications and updates, and record of dates for all file revisions.

Complete original electronic data sets are archived on 40GB backup tapes via an internal tape drive with MS Windows 2000 Server software. Electronic data are backed up on a daily basis Monday through Friday of each work week. The weekly tapes in use are stored at an off-site location to prevent loss due to a disaster such as fire or flood. These tapes are maintained indefinitely until they are replaced by a new set of backup tapes. The original hard copies of field data sheets and laboratory reports are stored in binders at the ANRA offices for a minimum period of five years.

Data Handling, Hardware, and Software Requirements

ANRA has put into place an electronic data processing system consisting of a network with the following configuration:

System Design

ANRA utilizes standard, IBM compatible, desktop personal computers that utilize the MS Windows XP operating system. Software operated includes MS Office Pro, Corel WordPerfect Office 2000, Accounting Express 2008 and Front Page 2002.

ANRA utilizes MS Access 2007 as the primary database management software. ANRA's Water Quality Database has been developed according to CRP guidance and database structures in accordance with TSSWCB and TCEQ requirements.

Information Resource Management Requirements

Data will be managed in accordance with the *SWQM Data Management Reference Guide*, (TCEQ 2010).and applicable Basin Planning Agency information resource management policies. GPS equipment may be used as a component of the information required by the SLOC request process for creating the certified positional data that will ultimately be entered into the TCEQ's SWQMIS database. In lieu of entering certified GPS coordinates, positional data will be acquired with a GPS and verified with photo interpolation using a certified source, such as Google Earth or Google Maps. The verified coordinates and map interface can then be used to develop a new station location.

LDC and SELECT Data Analysis

Systems Design

BAEN uses laptop computers and desktop computers. The computers run Windows XP or Vista operating system. Software includes Microsoft® Word, Microsoft® Excel, Microsoft® Access, and a Statistical Analysis System database management system run through Windows XP operating system. All GIS related work will be performed using ArcGIS 9x.

Backup and Disaster Recovery

The personal computer drives are backed up on a weekly basis to the network server and on a monthly basis to an external hard drive for storage in a secure secondary location. In the event of a catastrophic systems failure, the drives can be used to restore the data in less than one day's time. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

Archives and Data Retention

Original data recorded on paper files are stored for at least five years. Electronic data are stored on hard drives in climate-controlled, fire-resistant storage areas on the TAMU campus.

SELECT

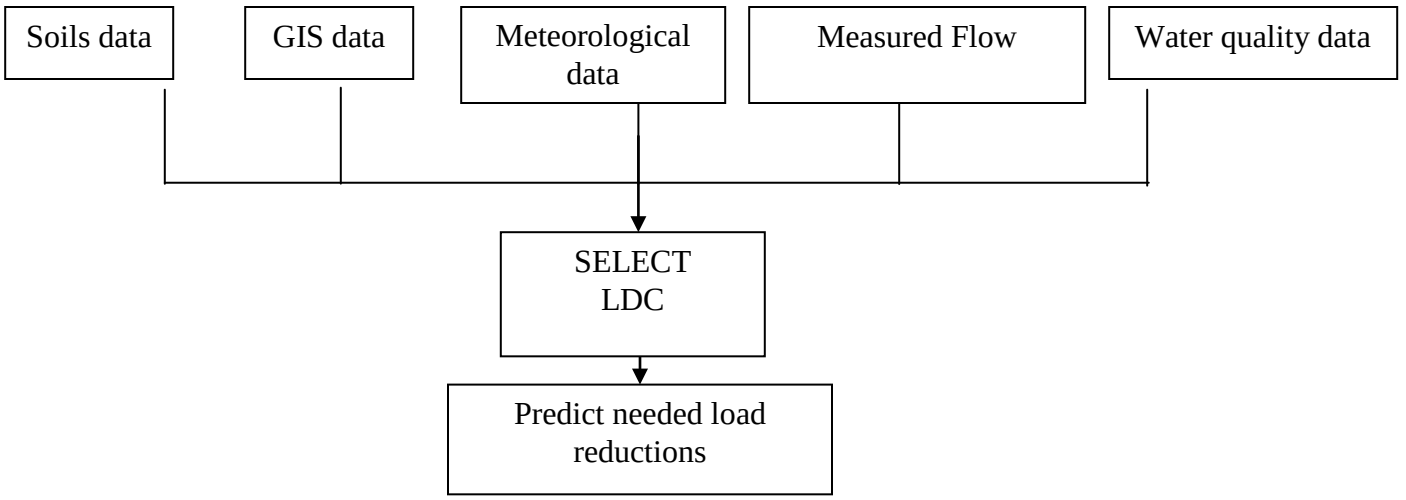


Figure B10.1. Information Dissemination Diagrams

RUAA

See Appendix H for the Field Data Sheets, Laboratory Data Sheets, and RUAA forms.

BST Analysis

Laboratory Data

Once the samples are received at SFASU SPWAL, samples are logged and stored at 4ºC until processed. The COC will be checked for number of samples, proper and exact ID number, signatures, dates, and type of analysis specified. SFA will be notified if any discrepancy is found and proper corrections made. The COC and accompanying sample bottles are submitted to the SFASU SPWAL analyst, with relinquishing and receiving personnel both signing and dating the COC. Processed samples will be stored at SFASU SPWAL in a refrigerator or freezer (depending upon sample type) until shipment of samples to SAML is arranged. Samples will be transported with COC, with relinquishing and receiving personnel both signing and dating the COC. All COC 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® operating system. The project spreadsheet will be maintained on the computer's hard drive, which is also simultaneously saved in a network folder. Data manually entered in the database will be reviewed for accuracy by the SCSC Project Lead or TWRI PM/QAO 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 COCs and bacteriological records related to QA/QC of bacteriological procedures will be housed at the SAML. 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.

Data Validation

Following review of laboratory data, any data 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 SCSC Project Leader, TWRI PM/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 Table D2.1.

Data Dissemination

At the conclusion of the project, the SCSC 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.

Section C1: Assessments and Response Actions

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

Table C1.1: Assessments and Response Actions

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status monitoring oversight	Continuous	ANRA, CES, SFASU, TWRI	Monitor project status, performance & records to ensure requirements are being fulfilled.	Report to TSSWCB PM in Quarterly Reports
Laboratory inspections	TBD by TSSWCB	TSSWCB	Analytical and quality control procedures in the lab	45 days to respond to TSSWCB w/ corrective actions
Technical systems audit	As needed	TSSWCB	Assess compliance with QAPP; review facility & data management as they relate to the project	45 days to respond to TSSWCB w/ corrective actions
Monitoring systems audit	TBD by TSSWCB	TSSWCB	Assess compliance with QAPP; review field sampling, facility & data management as they relate to the project	45 days to respond to TSSWCB w/ corrective actions

In-house review of data quality and staff performance to assure that work is being performed according to standards will be conducted by all entities. If review show that the work is not being performed according to standards, immediate corrective action will be implemented. CARs will be submitted to TSSWCB and documented in the project QPRs.

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, SWQM Procedures Manual, EP AREC SOPs, or Data Management Reference Guide. 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 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 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 Lead or PM of each respective entity. Audit reports and corrective action documentation will be submitted to the TSSWCB with the Progress Report.

Section C2: Reports to Management

Quarterly progress reports developed by the PM and Project Co-Leaders will note activities conducted in connection with the water quality modeling project, items or areas identified as potential problems, and any variations or supplements to the QAPP. CAR forms will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference by all project personnel and at TWRI and disseminated to individuals listed in section A3. 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.

If the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problem. CARs will be filled out to document the problems and the remedial action taken. Copies of CARs will be included with the project's quarterly reports. These reports will discuss any problems encountered and solutions made. These reports are the responsibility of the QAO and the PM and will be disseminated to individuals listed in section A3.

Task reports will be developed for the major project tasks (Land Use/Land Cover Update, Surface Water Quality Monitoring, RUAA, BST Analysis and Modeling Analysis). These individual task reports will be turned into Technical Reports by TWRI and hosted on the project website.

The final report for this project will be a completed watershed protection plan entitled "Attoyac Bayou Watershed Protection Plan." This document will be a culmination of the work conducted under this project and QAPP and will include information from technical reports that detail their respective tasks. The WPP and Technical Reports will be submitted as final project deliverables.

Section D1: Data Review, Validation and Verification

Land Use/Land Cover Update

This project will use NAIP 2009 winter imagery to conduct a general land cover inventory for each watershed. Ancillary data consisting of field surveys, available photography and existing vegetation maps will be used to classify vegetation and label distinct spectrally clustered polygons on the imagery. LULC classification will follow the methods and quality control standards outlined in this QAPP (Section A7). The project has a goal of achieving 80 percent accuracy in the overall classification of LULC. The coverage will include 5 watersheds in Texas with a minimum mapping unit of two acres. An independent set of ground reconnaissance data will be obtained to conduct the accuracy assessment analysis. Ground reconnaissance data will be reviewed and validated as outlined in Table D1.1.

Table D1.1. Ground Control Point Data Review, Validation and Verification Criteria

Data Element	Reviewed By	Validation Criteria
Coordinate Data	CES	Consistent with Sampling Process Design
Coordinate Data	CES	GPS Mode Matches Field Log & GPS Internal Data
Coordinate Data	CES	Default Settings Match GPS Internal Data
Coordinate Data	CES	Standard Deviation below 3 Meters for Acceptance
Coordinate Data	CES	Good Fit when Data Plotted against Known Locations
Coordinate Data	CES	Meets National Map Accuracy Standards
Metadata	CES	Meets EPA Guidelines for Metadata Documentation

Because of inherent technological, logistical, and financial constraints (Section B6), it is possible that the accuracy goal may not be achieved for all LULC classes. However, accuracy assessment will be essential for validating the final LULC map and providing the user with a measure of reliability. Only those data that are supported by appropriate quality control will be considered acceptable for use.

The procedures for verification and validation are described in Section D2, below. CES is responsible for ensuring that data are properly reviewed, verified, and submitted in the required format for the project. Finally, the TWRI QAO is responsible for validating that all data collected meet the data quality objectives of the project and are suitable for reporting.

All data obtained will be reviewed, validated, and verified against the data quality objects outlined in Section A7, “Quality Objectives and Criteria for Model Inputs / Outputs.” Only those data that are supported by appropriate QC will be considered acceptable for use.

The procedures for verification and validation are described in Section D2, below. CES is responsible for ensuring that data are properly reviewed, verified, and submitted in the required format for the project database. Finally, the TWRI QAO is responsible for validating that all data collected meet the DQOs of the project and are suitable for reporting.

Surface Water Quality Monitoring

All field and laboratory data will be reviewed and verified for integrity and continuity, reasonableness, and conformance to project requirements, and then validated against the project objectives and measurement performance specifications which are listed in Section A7. Only those data which are supported by appropriate quality control data and meet the measurement performance specifications defined for this project will be considered acceptable, and will be reported to the TSSWCB for entry into SWQMIS.

LDC and SELECT Data Analysis

The procedures for verification and validation of data used in water quality modeling analysis are described in Section D2, below. The BAEN Project Co-Leader is responsible for ensuring that data are properly reviewed, verified, and submitted in the required format for the project database. Finally, the TWRI PM/QAO is responsible for validating that all data collected meet the DQOs of the project and are suitable for reporting.

RUAA

Field data recorded will be checked for transcription errors and calculations of flow readings will be verified for accuracy.

BST Analysis

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

The procedures for verification and validation of data used in BST analysis are described in Section D2. The SCSC Project Co-Lead/SAML Director is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The TWRI PM/QAO 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 PM. Finally, the TWRI PM/QAO is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

Section D2: Validation Methods

Land Use/Land Cover Update

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. CES is 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 all raw data and electronically generated data. The field data will be verified and validated as described in Table D2.1.

Verification, validation and integrity review of LULC data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by CES. The LULC data generated are evaluated against ground control points and project specifications and are checked for errors. Potential outliers are identified by examination for unreasonable data. If a question arises or an error or potential outlier is identified, then issues will be resolved through mutual consultation between CES, TWRI QAO, and TSSWCB PM. Issues which can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, CES consults with the TWRI PM to establish the appropriate course of action.

The final versions of the land cover maps and the accuracy assessment report will be peer reviewed prior to its release to the TSSWCB and the public. Prior to release, CES has responsibility for reviewing all data and verifying that final products achieved QAPP-defined goals for accuracy, completeness and acceptance criteria. The final version of each land cover map will be conveyed to users as digital GIS files in ARC/INFO format on CD-ROM disks. Hard copy maps will also be provided free to the TSSWCB as needed.

The final element of the validation process is consideration of any findings identified during assessments or audits conducted by the TWRI or TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, CES in coordination with the TWRI QAO validates that the data meet the data quality objectives of the project and are suitable for reporting to the TSSWCB.

Surface Water Quality Monitoring

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 to the project task. The data review tasks to be performed by field and laboratory staffs are listed in the first column of Table D2.1. 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

manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented. If an issue cannot be corrected, the task manager consults with the higher level project management to establish the appropriate course of action, or the data associated with the issue are rejected and not reported to the TSSWCB for submission to TCEQ for storage in SWQMIS. Field and laboratory reviews, verifications, and validations are documented.

Table D2.1. Data Review Tasks

Data to be Verified	CES	SFASU	ANRA	SCSC	TWRI	TSSWCB
LULC Data Coordination: verify data are correct and fall w/in standards	L					PM
Sample documentation complete; samples labeled	L	L	PM	LM		
Field QC samples collected for all analytes as prescribed in the TCEQ SWQM Procedures Manual		L	PM			
Standards and reagents traceable		LM	LM	LM		
Chain of custody complete/acceptable		LM	LM	LM		
NELAC Accreditation is current			LM	LM		
Sample preservation and handling acceptable			LM	LM		
Holding times not exceeded		LM	LM	LM		
Collection, preparation, and analysis consistent with QAPP and guidance documents	L	L/LM	PM/LM	L/LM	PM	PM
Bacteriological records complete		L/LM	LM	LM		
QC samples analyzed at required frequency		LM	LM	LM		
QC results meet performance and program specifications		LM	PM/LM	L/LM		
Results, calculations, transcriptions checked		LM	PM/LM/DM	L/LM		
All laboratory samples analyzed for all parameters		LM	LM	LM		
Nonconforming activities documented	L	L/LM	PM/LM	L/LM	PM	PM
Outliers confirmed and documented; reasonableness check performed		LM	DM			
Data properly formatted for SWQMIS inclusion and checked for errors			DM			PM

L: Leader LM: lab manager PM: project manager DM: data manager

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 as specified in Table D2.1 is performed by the ANRA Project/Data Manager and QAO. 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.

The Data Review Checklist (See Appendix F) covers three main types of review: data format and structure, data quality review, and documentation review. The Data Review Checklist is

transferred with the water quality data submitted to the TSSWCB to ensure that the review process is being performed.

Another element of the data validation process is consideration of any findings identified during the monitoring systems audit conducted by the TSSWCB QAO. 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 ANRA PM validates that the data meet the data quality objectives of the project and are suitable for reporting to TSSWCB and subsequently TCEQ.

If any requirements or specifications of the QAPP are not met, based on any part of the data review, the responsible party should document the nonconforming activities and submit the information to the ANRA Data Manager with the data. This information is communicated to the TSSWCB by the ANRA in the Data Summary (See Appendix F).

LDC and SELECT Data Analysis

There is no validation and calibration for the SELECT model or LDC as they are data processors.

RUAA

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and RUAA specifications as described in TCEQ *Procedures for a Comprehensive RUAA and a Basic RUAA Survey* (TCEQ 2009).

Data review, verification, and validation will be performed using self-assessments and peer and management review as appropriate to the project task.

BST Analysis

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 COC 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 PM/QAO to establish the appropriate course of action, or the data associated with the issue are rejected.

The SCSC Project Lead, with assistance from the TWRI PM/QAO, is responsible for validating that the verified data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data for anomalies. The SCSC Project Lead may designate other experienced water quality experts (ANRA PM or SFASU Project Co-Lead) familiar with the waterbodies 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 PM/QAO or TSSWCB QAO. 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/QAO validates that the data meet the DQOs of the project and are suitable for reporting to the TSSWCB.

Section D3: Reconciliation with User Requirements

Land Use/Land Cover Update

The GPS Reconnaissance Survey results and products will be evaluated against the Data Quality Objectives established and user requirements to determine if any reconciliation is needed. Reconciliation concerning the quality, quantity or usability of the data will be reconciled with the user during the data acceptance process. Types of reconciliation may include reduction in the scope of the project in terms quality or quantity of data produced in meeting partial user requirements.

Once the final version of each LULC Map is produced, the TSSWCB Project Manager will review the product and the accuracy assessment report to determine if they fall within the acceptance limits as defined in this QAPP. Completeness will also be evaluated to determine if the completeness goal for this project has been met. If data quality indicators do not meet the project's requirements as outlined in this QAPP the data may be returned for revisions.

These data, and data collected by other organizations, will subsequently be analyzed and used for watershed assessment, TMDL and watershed plan development, and modeling activities. 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.

Surface Water Quality Monitoring

Data produced in this project, and data collected by project personnel will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be used in the development of the Attoyac Bayou WPP and will be submitted to TCEQ assessment purposes and use in the *Texas Water Quality Inventory and 303(d) List* in accordance with TCEQ's *Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data.*(2004). Data which do not meet requirements will not be submitted to SWQMIS nor will be considered appropriate for any of the uses noted above.

LDC and SELECT Data Analysis

The SELECT modeling framework developed for this project will be used to evaluate bacteria loading in the Attoyac Bayou watershed. It will provide information pertaining to watershed characteristics and to the prediction of possible pollution, the sources of this pollution and will provide critical information to assist in identifying management practices to prevent pollution loading in area streams. This, in turn, will be useful for incorporation in the WPP being developed this project.

The LDC framework utilized for this project will be used to evaluate bacteria and ammonia loading in relation to flow regimes in Attoyac Bayou. This approach will utilize flow data collected during this project and pair them with real bacteria and ammonia water quality data to illustrate times when loadings exceeds standards. These analyses will aid in targeting water

quality best management practices recommendations to the most likely areas of bacteria and nitrate impairment.

RUAA

Data produced in this project, and data collected by project personnel will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be used in the development of a RUAA report for the Attoyac Bayou and its assessed tributaries and will be submitted to TCEQ for appropriate use assessment purposes.

BST Analysis

Data produced by this project will be evaluated against the established DQOs and user requirements to determine if any reconciliation is needed. Reconciliation concerning the quality, quantity or usability of the data will be reconciled with the user during the data acceptance process. CARs will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the DQOs of the project and the informational needs of water quality agency decision-makers and watershed stakeholders.

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only BST data that has met the DQOs described in this QAPP will be reported and included in the final project report. Data and information produced thru this project will provide needed information pertaining to watershed characteristics, potential sources of pollution and will aid in the selection of BMPs to address identified water quality issues. Ultimately, stakeholders will use the information produced by this project for the development of appropriate measures to address water quality concerns in the study area. Information produced by this project will be for watershed decisions; namely the development of a WPP.

References

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.

Casarez, E.A, S.D. Pillai, J. Mott, M. Vargas, K. Dean and G. Di Giovanni,2007. "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

Cleland, B. 2003. TMDL Development from the "bottom up" – Part III: Duration Curves and wet-weather assessments. America's Clean Water Foundation, Washington, DC.

Dick, L.K., A.E Bernhard, T.J. Brodeur, J.W. Santo Domingo, J.M. Simpson, S.P. Walters and K.G. Field. "Host distributions of uncultivated fecal *Bacteroidales* bacteria reveal genetic markers for fecal source identification." Appl Environ Microbiol 71(6): 3184-3191.

Field, K.G, E.C. Chern, L.K. Dick, J. Fuhrman, J. Griffith, P.A. Holden, M.G. LaMontagne, J. Le, B. Olson and M.T. Simonich. "A comparative study of culture-independent, library-independent genotypic methods of fecal source tracking." J Water Health 1(4): 181-194.

Jones, C.A, K. Wagner, G. Di Giovanni, L. Hauck, J. Mott, H. Rifai, R. Srinivasan, G. Ward. 2009. "Bacteria Total Maximum Daily Load Task Force Final Report." Texas Water Resources Institute. Technical Report 341.

Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005.

Stiles, T.C., 2001. A simple method to define bacteria TMDLs in Kansas. KS Dept. of Health and Environment. Topeka, KS. http://www.wef.org/pdffiles/TMDL/Stiles.pdf (last accessed, 9/12/2006).

TCEQ. 2004. "Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data." http://www.tceq.state.tx.us/assets/public/compliance/monops/water/04twqi/04_guidance.pdf

TCEQ. 2009. "Recreational Use-Attainability Analyses (RUAAs): Procedures for a Comprehensive RUAA and a Basic RUAA Survey." http://www.tceq.state.tx.us/assets/public/permitting/waterquality/attachments/stakeholders/recUAAproceduresMay2009.pdf

TCEQ. 2010. "Surface Water Quality Monitoring Data Management Reference Guide." http://www.tceq.state.tx.us/assets/public/compliance/monops/water/wdma/dmrg/dmrg_complete.pdf

TCEQ SOP,V1 - TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue, 2012 (RG-415)

USEPA. 2001. "Environmental Investigations Standard Operating Procedures and Quality Assurance Manual." USEPA Region 4, November 2001. http://www.orau.org/ptp/PTP%20Library/library/EPA/samplings/eisopqam.pdf

USEPA. 2006. "*Escherichia coli* in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia coli* (modified m-TEC) Agar." EPA-821-R-06-011. July 2006.

USEPA. 2007a. "An Approach for Using Load Duration Curves in the Development of TMDLs." EPA 841-B-07-006. http://www.epa.gov/owow/tmdl/duration_curve_guide_aug2007.pdf

USEPA. 2007b. "Options for Expressing Daily Loads in TMDLs." Draft. http://www.epa.gov/owow/tmdl/draft_daily_loads_tech.pdf

USEPA. 2007c. "Geospatial Metadata Technical Specification." Version 1.0, January, 2007.

USEPA. 2008. "Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality." EPA/600/R-08/065. http://www.epa.gov/nrmrl/pubs/600r08065/600r08065.pdf

United States Environmental Protection Agency (USEPA) Methods for Chemical Analysis of Water and Wastes. Manual #EPA-600/4-79-020

United States Environmental Protection Agency (USEPA) Manual #EPA-821-R-9S-027

Versalovic, J., M. Schneider, F.J. de Bruijn and J.R. Lupski. 1994. Genomic fingerprinting of bacteria using repetitive sequence based PCR (rep-PCR). Meth.Cell. Mol. Biol. 5: 25-40

Walkowiak, M.A., ed. 2006. *ISCO Open Channel Flow Measurement Handbook.* 6th ed. Lincoln, NE.

Zeckoski, R.W., B.L. Benham, S.B. Shan, M.L. Wolfe, K.M. Brannan, M. Al-Smadi, T.A. Dillaha, S. Mostaghimi, and C.D. Heatwole, 2005. "BSLC: A tool for bacteria source characterization for watershed management." Transactions of ASAE, 21(5): 879-889.4

This Page Left Blank Intentionally

APPENDIX A

Corrective Action Report

This Page Left Blank Intentionally

Corrective Action Report
SOP-QA-001
CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

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

Possible causes:
_____
_____
_____

Recommended Corrective Actions:
_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:
_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Program Manager:_____

TWRI Quality Assurance Officer:_____

TSSWCB Quality Assurance Officer:_____

This Page Left Blank Intentionally

APPENDIX B

Land Use/Land Cover Field Survey Form

This Page Left Blank Intentionally

Land Use/Land Cover Field Survey Form

Name: _____ **Date:** _____

Agency: _____ **Watershed:** _____

Site Name: _____

Point Number: _____

UTM Coordinates: _____

or

Latitude/Longitude: _____

Land Use/Land Cover: Use descriptions presented in Section A5 to determine appropriate LULC for this point

Developed Open Space	_____	Forested Land	_____
Developed Low Intensity	_____	Near Riparian Forested Land	_____
Developed Medium Intensity	_____	Mixed Forest	_____
Developed High Intensity	_____	Rangeland	_____
Open Water	_____	Pasture/Hay	_____
Barren Land	_____	Cultivated Crops	_____

How confident are you of your LULC assessment at this site?

_____ High confidence _____ Medium confidence _____ Low confidence

Comments:

This Page Left Blank Intentionally

APPENDIX C

Surface Water Quality Monitoring Field Data Sheet

This Page Left Blank Intentionally

Surface Water Quality Monitoring
Field Data Sheet

Stephen F. Austin State University
P.O. Box 6109, SFA Station
Nacogdoches, TX 75962-6109
(936) 468-2469

Sample Location: _____

Station ID: _____ **Date Collected:** _____

Sample Matrix: Water / Fecal **Time Collected:** _____

Collector(s) Name/Signature: _____

Sample Type: Routine / Storm **Sample Depth:** _____

Field Tests and Measurements:				Parameters Collected:		
	pH (standard units)	00400		E. coli (IDEXX)		Total N
	water temperature °C	00010		E. coli (mTEC)		NNN
	Dissolved Oxygen (mg/L)	00300		TSS		Total P
	Specific Conductance (µS/cm)	00094		Diss. Ortho-P		
	Instant. Stream Flow (cfs)	00061		Ammonia-N		Field Split
Field Observations						
	01351 - Flow Severity (1 - no flow, 2 - low, 3 - normal, 4 - flood, 5 - high, 6 - dry)					
	89835 - Flow measurement method (1-gage, 2-electric, 3-mechanical, 4-weir/flume, 5-doppler)					
	72053 - Days since last significant rainfall					
	89966 - Present weather (1 - clear, 2 - partly cloudy, 3 - cloudy, 4 - rain, 5 - other)					
	74069 - Stream flow estimate (cfs) *Required measurements to calculate flow estimates					
	Stream width (feet)*					
	Averagae depth of stream (feet)*					
	Distance object travels (feet)*					
	Time for object to travel distance (seconds)*					

Note: Instantaneous stream flow is preferable to a stream flow estimate

Comments:

This Page Left Blank Intentionally

APPENDIX D

Chain of Custody Records

Chain of Custody Record

Project:	Collecting Entity:
Development of a Watershed Protection Plan for Attoyac Bayou TSSWCB Project #09-10	Stephen F. Austin State University P.O. Box 6109, SFA Station Nacogdoches, TX 75962-6109 Contact: Matthew McBroom: 936.468.2469
Name and signature of collector:	Receiving Entity: Angelina Neches River Authority P.O. Box 387 Lufkin, TX 75901 Contact: Brian Sims: 936.633.7527

Station ID	Sample ID	Media Code	Sample Type (Routine / Storm)	Number of Containers	Collection Date	Time	Container Type						Container Types:		
							S	H	H	H	H	H	H: Precleaned High Density Polyethelyene		
							Preservative						SP: Sterile Polyethelyene container		
													Preservatives:		
							Requested Anaylsis						1: ice	2: H2SO4	3: Sodium Thiosulfate
							E. coli (IDEXX)	Total Suspended Solids	Ammonia - N, Total	Nitrate/Nitrite - N	Dissolved Ortho - P	Total P	Sample Receipt Notes:		
													OK: all samples 100% correct (labels, COCs, shipping)		
													1: not on ice	2: incorrectly labeled	3: COC incorecct
													4: bottle leaking	5: other (note in comments section)	
													Sample Receipt Notes: (Report any issues here: ie. Not on ice, improper label, damaged bottle, leaky bottle, etc.)		
													1	2	3
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													
		water													

Relinquished by: (print name)	Date:	Time:	Received by: (print name)	Date:	Time:
1					
2					
3					

Special Comments:

Laboratory Comments:

Chain of Custody Record

Project:	Collecting Entity:
Development of a Watershed Protection Plan for Attoyac Bayou *TSSWCB Project #09-10*	Stephen F. Austin State University P.O. Box 6109, SFA Station Nacogdoches, TX 75962-6109 Contact: Matthew McBroom: 936.468.2469
Name and signature of collector:	Receiving Entity:
	Stephen F. Austin State University P.O. Box 6109, SFA Station Nacogdoches, TX 75962-6109 Contact: Leon Young: 936.468.4500

Container Type						Container Types:
SSB	SSB	SC	PD			SSB: sterile sample bag (whirl-pak)
Preservative						SC: Sterile Container
						PD: 50mm x 9 mm petri dish
Requested Anaylsis						Preservatives:
Media Required:						1: ice 2: GITC Buffer
water	supor filter	feces	mTec agar			Sample Receipt Notes:
E. coli (mTec)	Bacteroidales	Fecal Specimen	E. coli water isolates			OK: all samples 100% correct
						1: not on ice 2: incorrectly labeled 3: COC incorecct
						4: bottle leaking 5: other (note in comments section)

Station ID	Sample ID	Media Code	Sample Type (Routine / Storm)	Number of Containers	Collection Date	Time	Sample Receipt Notes: 1	2	3
		water							

Relinquished by: (print name)	Date:	Time:	Received by: (print name)	Date:	Time:
1					
2					
3					

Special Comments:

Laboratory Comments:

This Page Left Blank Intentionally

APPENDIX E

BST STANDARD OPERATING PROCEDURES

E-1: Archival of *Escherichia coli* Isolates 123
E-2: ERIC-PCR of *Escherichia coli* 125
E-3: RiboPrinting of *Escherichia coli* 130
E-4: *Bacteroidales* PCR 147

This Page Left Blank Intentionally

E-1: Archival of *Escherichia coli* Isolates

Note: All handling of cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. These isolates should be from colonies which have been plated for purity several times and lab personnel are confident purity has been achieved.

1. Select a well-isolated colony of purified *E. coli*. (Examine the cultures using a long-wave handheld UV lamp, colonies will fluoresce).

2. Using a bacteriological loop, transfer the colony to a labeled sterile cryovial containing 1 mL of tryptone soy broth (TSB) with 20% reagent grade glycerol.

3. Firmly cap the cryovial and verify that the cells have been resuspended by vortexing for several seconds; then plunge into liquid nitrogen until frozen.

4. Immediately transfer to a cryostorage box and place in -70 to -80°C freezer. Cultures may be stored for several years under these conditions.

5. To recover cultures from frozen storage, remove the cultures from the freezer and place the cryovials in a freezer block. *Do not allow cultures to thaw.*

    a. Using a bacteriological loop, scrape the topmost portion of the culture and transfer to growth medium, being careful not to contaminate the top or inside of the vial. Invert and incubate plates at 35 to 37°C for 20 to 24 h.

    b. Reclose the cryovial before the contents thaw and return to the freezer.

Casarez, E. A.; Pillai, S. D.; Mott, J.; Vargas, M.; Dean, K.; Di Giovanni, G. D. (2007). "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

This Page Left Blank Intentionally

E-2: ERIC-PCR of *Escherichia coli*

1. Select isolated colonies from overnight cultures of *E. coli* isolates on Brain-Heart Infusion (BHI) plates.

2. Transfer colonies using a 1 µL loop to a sterile microfuge tube containing 100 µL of sterile molecular grade water; vortex briefly to suspend cells.

3. Prepare sufficient PCR Master Mix for samples, including one blank per 10 samples to account for volume loss due to repeat pipetting. Prepare Master Mix for each sample as noted below. One full PCR batch on the MJ Research Cycler 48 well-plate will have 46 samples, *E. coli* QC101, and a no template control.

   ERIC-PCR Master Mix – 24 samples + 2 blanks, prepare X 2 for full 48-well plate

MASTER MIX	Amt (uL)	Final Calc	Final Units
dH2O	**819**		
10X PCR buffer I w Mg	**130**	1	X (1.5 mM)
20 mM dNTP	**13**	200	uM each
ERIC Primer Mix	**130**	600	nM each
BSA (30 mg/ml)	**65**	1.5	ug/uL
AmpliTaqGold (Units)	**13**	2.5	Units/rxn

4. Dispense 45 µl of Master Mix for each sample into the appropriate well of PCR plate.

5. Briefly vortex cell suspensions, then add 5 µl of each cell suspension to the appropriate PCR well.

6. Carefully seal plate using an adhesive PCR cover.

7. Load the plate into the thermal cycler and run under the "ERIC-PCR" program with the following cycling conditions:

   a. Initial denaturation at 95°C for 10 min

   b. 35 Cycles:
      i. Denaturation at 94°C for 30 sec
      ii. Annealing at 52°C for 1 min
      iii. Extension at 72°C for 5 min

   c. Final Extension at 72°C for 10 min

8. Store completed reactions at -20°C until analyzed by gel electrophoresis.

9. Prepare a 250 mL, 2% agarose gel using a 500 mL bottle. Add 250 mL of 1 X Tris/Borate/EDTA (TBE) buffer and 5.0 g agarose. Microwave until agarose is fully dissolved, tighten cap and let cool 1 to 2 minutes, then pour agarose into casting tray with 30-tooth, 1 mm thick comb.

10. Allow gel to solidify for approximately 30 minutes on the bench, then without removing comb place in Ziploc bag and solidify overnight in the refrigerator. The next day carefully remove comb, transfer to gel tank containing pre-cooled 1X TBE buffer. Replace TBE in gel tank after it has been used twice.

11. The following items will be needed for electrophoresis:

    100 bp ladder (0.33 µg/10 µL) (1500 µL final, enough for 150 lanes)

    200 µL Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µL 100 bp ladder (add reagents below to a full tube of marker)

    300 µL 6X ERIC-PCR loading buffer (see recipe below)

    150 µL 10X PCR buffer

    850 µL molecular grade water

    Store in cold room

    6X ERIC-PCR Loading Buffer

    25 mg bromphenol blue (0.25%)

    1.5 g ficoll 400 (15%)

    Add molecular grade water to 10 mL, divide into 1 mL aliquots and freeze, the aliquot currently being used can be stored in the cold room

    ERIC-PCR Blank

    100 µL 10X PCR buffer

    200 µL 6X ERIC-PCR loading buffer

    900 µL molecular grade water

    Store in cold room

Ethidium Bromide Stain (0.5 µg/mL)

1250 mL 1X TBE

62.5 µL ethidium bromide (Sigma, 10 mg/mL)

Store covered at room temp, can use up to 5 times by adding 10 µL ethidium bromide each additional use

12. Mix 10 µL of 6X ERIC-PCR Loading Buffer to each PCR well and mix with pipette tip.

13. Load the gel in the cold room as follows (max. of 23 samples + QC101 + NTC per gel):

    a. Load 10 µl of 100 bp ladder (0.33 µg) into the first lane

    b. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    c. Load 10 µl of 100 bp ladder (0.33 µg)

    d. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    e. Load 10 µl of 100 bp ladder (0.33 µg)

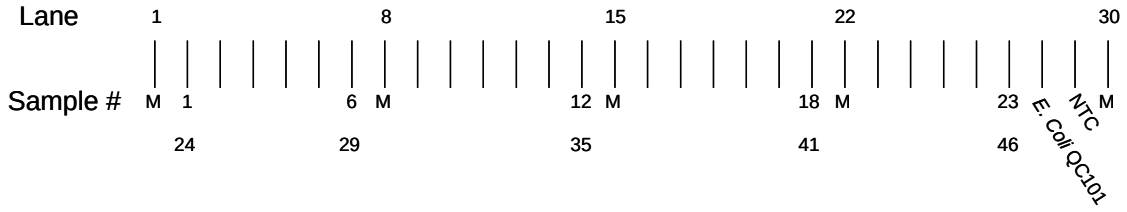
    f. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    g. Load 10 µl of 100 bp ladder (0.33 µg)

    h. Load 10 µl of sample ERIC-PCR reactions into next 5 lanes

    i. Load PCR Batch *E. coli* QC101 and NTC into next 2 lanes

    j. Load 10 µl of 100 bp ladder (0.33 µg)



If running a gel with fewer samples, follow steps above until last sample, followed by *E. coli* QC101, NTC and ladder, then load ERIC-PCR Blank into remaining lanes on gel.

14. Start electrophoresis power supply set at 100 volts, run for 1 hour.

15. Stop power supply, set time to “000”, set voltage to 200 and start circulating pump at setting #2, run for 4 hours.

16. After electrophoresis, stain gel in Ethidium Bromide Stain for 20 minutes with agitation (save stain, see Step 13).

17. Destain gel for 10 minutes in 1X TBE buffer. Save destain, can be used 3 times then discard.

18. Follow Gel Imager SOP for image capture. Save digital photograph as a TIFF file (default) and print a hardcopy for notebook.

Casarez, E. A.; Pillai, S. D.; Mott, J.; Vargas, M.; Dean, K.; Di Giovanni, G. D. (2007). "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

This Page Left Blank Intentionally

E-3: RiboPrinting of *Escherichia coli*

Storing and Handling Disposables

Check the lot expiration date on each label for details and rotate the stock to optimize use.

Heating membrane and probe (MP) Base

After storage and the temperature changes that occur during shipment, the oxygen in the buffer loaded in the MP base may need to be removed before use. This is called degassing and is accomplished by heating the base pack overnight in your incubator.

To degas buffer:

1. Place enough MP base packs for the next day's production in their storage pouches in an incubator set at 37°C.

2. Allow the base pack to degas for 16 to 24 hours prior to loading in the characterization unit. You may do this while you are incubating samples, since the base packs are sealed in their pouches. This procedure allows you to start a batch immediately at the beginning of the next shift.

3. If you do not use the heated base packs, you can return them to storage and reuse them. These base packs should be heated again before reuse since temperature cycling affects oxygen content in the buffer.

Preparing Lysing Agent (for *Staphylococcus* and lactic-acid bacteria only)

Lysing agent (A and B) is shipped frozen and must be stored at -20°C. Lysing agent must be thawed before use. This only takes about 5 minutes. If the lysing agent will not be used again for more than 2 hours, the material should be returned to the freezer. Lysing agent can be re-frozen several times with no effect on performance.

Sample Preparation Procedures

1. Incubate and Inspect the Samples

Use BHI agar plates prepared within the last 30 days. Do not use plates that appear dry or dehydrated. Such plates can cause problems when you attempt to "pick" the colonies for use in the RiboPrinter® system.

1. Using a pure isolated colony as the source, streak BHI agar plates heavily in the upper portion of the plate to create a lawn. Streak the remainder of the plate lightly to create single colonies.

2. Follow standard laboratory techniques. Heat plates for 18 to 30 hours in a humidified incubator at 37 °C.

2. Transfer Sample Buffer to Intermediate Tubes

a) Locate the 250 mL twist-top bottle of sample buffer supplied in Pack # 1. Install the twist cap.

b) Transfer about 5 mL of buffer to a sterilized disposable 15 mL intermediate working tube.

3. Add sample buffer to microcentrifuge tubes

1. Place a sterile 0.65 mL microfuge tube in each of the eight holes in the lower row of the sample preparation rack.

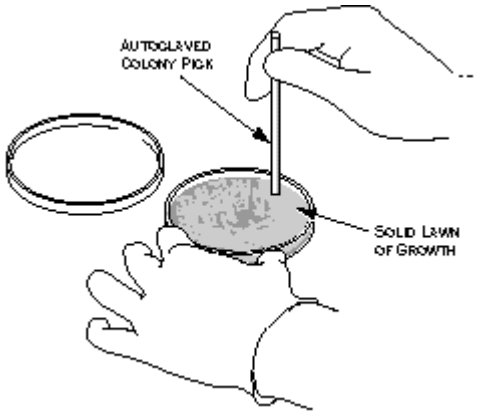
2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.

   For Gram positive samples (e.g. *S. aureus* and *L. innocua* QC strains), add 40 µL of sample buffer.

3. Close the lids on the tubes.

4. Harvest the Samples

1. Using autoclaved colony picks and making certain not to gouge the agar, carefully place the pick into one of the single colonies or the lawn. You need a sample area at least equal to that of the bottom of the colony pick. In most cases you will need to harvest from the lawn area of the plate. If you are working with large colonies, a single colony will be adequate.



2. <u>For Gram negative samples (e.g. *E. coli*), perform 1 pick placed into 200 µL of sample buffer.</u>

CAUTION! Do not try to use the same pick twice on a plate. You need to harvest only enough sample to cover the bottom surface of the pick. Make sure the end of the pick is flat, if not, use a different pick.

CAUTION! Do not overload the harvesting pick. Collect only enough sample to cover the base of the pick. Over sampling will cause inaccurate results. Over sampling is a particular problem with *Staphylococcus*.

5. Mix the Samples

WARNING! *Perform sample preparation using a Class 2 biological safety cabinet since aerosols may be formed during mixing of the samples.*

1. Making certain not to touch the sample end of the pick, place the pick into one of the filled sample tubes.

2. While holding the tube with the open end facing away from you, carefully attach the pick to the hand-held mixer. The fit of the pick in the coupling will be loose.

WARNING! Do not turn on the mixer unless the pick is inside the sample tube and below the surface of the liquid. Turning the unit on at other times will cause the sample to aerosolize and may cause contamination.

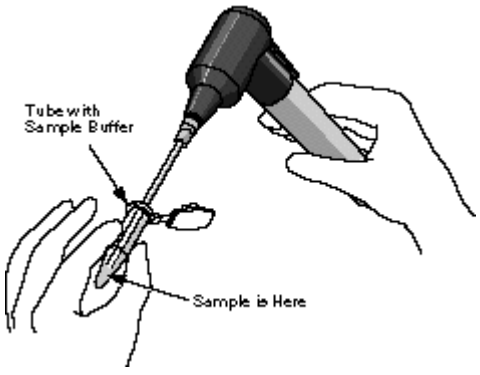
3. Press the ON lever on the mixer for about 5 seconds.

4. Release the lever and carefully remove the colony pick. The sample liquid should appear turbid.

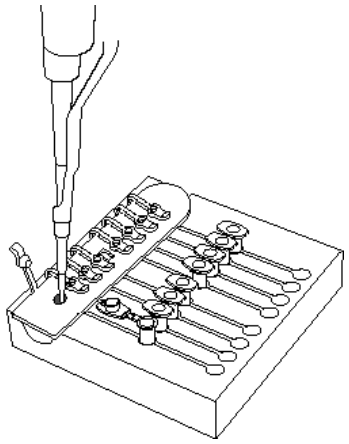
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.

6. Cap the sample tube.

7. Move the tube to the top row of the sample preparation rack. This indicates that the tube is filled.



6. Transfer the Samples to the Sample Carrier



1. Open the lid covering the first well of the sample carrier.

2. Using a 100 µL pipetter, pipette 30 µL of sample from the microcentrifuge tube into the well.

3. Close the lid cover for the well.

4. Repeat for remaining samples using a new pipet tip for each sample.

CAUTION! Transfer the sample carrier to the Heat Treatment Station within 2 hours. If you wait longer than 2 hours, you will have to discard the sample carrier and begin again for this batch.

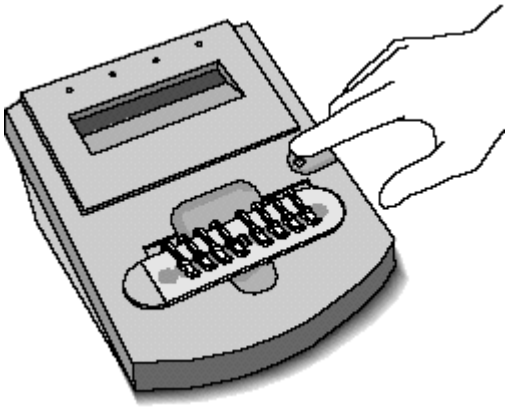
6. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).

7. Write down the name or code you use to identify the sample and the well number in the sample carrier for each sample using a sample log sheet.

7. Place the Sample Carrier in the Heat Treatment Station and Process the Sample Carrier

1. Place the sample carrier into the Heat Treatment Station. The display on the Heat Treatment Station will show **Insert**, if power is available. If the display is blank, make certain that the power cord on the back of the station is properly connected.

   After you insert the carrier, the display shows **Press Button.**



2. Press the button on the Heat Treatment Station.

   The display shows **Warm up** and counts down from **10** while the station is warming up. The actual warm up cycle varies with the condition of the room and the heat treatment station. Normal time is about 4 minutes.

   When the station reaches operating temperature, the display changes to **Heat** and counts down from **13.** This represents each minute of heat treatment.

   The indicator message changes to **Cool.** The display counts down from **9**, indicating the minutes remaining in the cooling cycle**.** If necessary, you can remove the carrier as soon as the **Cool** message appears.

3. The heat treatment step is finished when the display shows **READY** and counts down from **90.** The display will flash and an audible beep will sound three times. The alarm will then beep once every 10 minutes until the sample is removed or 90 minutes elapses.

Caution! The heat-treated samples must be used within the 90-minute period at room temperature or they must be discarded. The heat-treated samples may be stored at this point (prior to adding Lysis Agents, if required) for 1 week at 4 °C, or for several months at -70 °C.

8. Add the Lysing Agents (for *Staphylococcus* and lactic-acid bacteria only)

1. Using a 10-µL pipetter and new tips for each addition, add 5 µL of Lysing Agents A and B to each sample. Note: this step is omitted for *E. coli* as it has no effect on ribopatterns. Lysing Agents were specifically developed for *Staphylococcus* and Lactic-Acid bacteria samples.

Caution! This step must be performed just prior (within 10 minutes) of loading the samples into the RiboPrinter and starting the run.

Creating and Loading a Batch

There are three options under the Operations menu for creating standard batches:

- *Eco*RI batches (VCA)
- *Pst*I batches (VCB)
- *Pvu*II batches (VCC)

You can also create special batches:

- Restriction Enzyme Flexibility batches
- **Substitute Enzyme batches (including *Hin*d III)**

From the Instrument Control Base Window:

1. Move the pointer to Operations and click with the mouse button. The Operations menu appears.

2. Move the pointer to Create Substitute Enzyme Batch and click with the mouse button.

3. Use the View menu to remove any optional items you do not wish to fill in. The system requires at least Sample Type and RiboGroup Library information for each sample. You cannot remove these options. The **Clear** option de-selects the **Use Default ID Libraries.** You will have to enter a DuPont ID and Custom ID library name for all samples. These become required fields and the system will make you enter data before you can save the information in this window.

CAUTION! If you change the display after you have entered information, you will lose all the information in the window. The window will redraw with a new blank display showing the items you have selected.

4. To enter information about the sample, click on the **View** button with the mouse button, then click on **Sample Items**. Click on the options you want to display.

5. Enter your initials and any comment you want to record about the batch.

6. Select the lot number fields and record for all reagents.

CAUTION! All fields must be completed or the system will not let you start processing the batch.

7. For each well in the sample carrier, choose the type (Sample or Control [QC Number]) from the Sample Type field. The system defaults to Sample.

8. Once you define the Sample Type as Sample, type in the name you actually want to use. This information will appear as Sample Label in the Data Analysis software screens.

9. You can change the RiboGroup library name if needed. Do this by clicking on the button next to the field with the mouse button. A pop up menu appears listing your choices. If you want to add a new library name, move the pointer to the line and click with the mouse button to get a cursor, then type in the new library name. Once you have saved this file, the new name will be added to the pop up list for future use. Do **NOT** change the DuPont ID field. If you select one of the QC strains, the system automatically enters QC in the DuPont ID and RiboGroup Library fields. Do not change these names. If you wish, you may enter a name for the Custom ID library.

10. Repeat for the other seven samples.

11. Click on Save and Submit Batch to Instrument.

Loading Disposables

Follow the screen prompts to load disposables and check the DNA Prep Waste. The icons on the window will flash red to tell you to remove and load an item. The screen prompts you about which Separation and Transfer chamber to use for the membrane and gel cassette. The LDD Pipette will move to physically block you from placing samples in the wrong chamber.

CAUTION! *Do not try to move the pipette manually. You will cause the system to lose the step count. This can result in the loss of batch data. If the pipette is blocking the S/T chamber that you are instructed to use, STOP. Call Customer Support.*

CAUTION! *Do not load disposables until you are prompted by the system. If you try to load them earlier, the alarm will sound as long as the doors are open. If you do load disposables ahead of time, the MP Base will be moved to the wrong position and you will not be able to begin processing the batch. You will not be able to move the MP base manually.*

1. Check the DNA Preparation Waste Container

1. The DNA Prep waste container must be visually checked before every batch. If the container looks nearly full (about 1 inch from the top), remove the container, unscrew the cap and empty into the liquid biohazard waste.

WARNING! Do not tip the DNA Preparation waste container when you remove it.

WARNING! Do not unscrew the cap from the DNA Preparation waste container if the fluid level has risen into the cap. First pour the excess waste liquid into the liquid biohazard waste.
WARNING! When replacing container make sure that the cap is properly threaded in place. If the cap is only partially threaded, it can snag the pipette during operation.

2. Load the Sample Carrier

1. Place the sealed carrier into the labeled slot on the far right of the characterization unit.

2. Push the sample carrier down firmly until it snaps into place.

CAUTION! Place the rounded edge of the sample carrier on your right as you view the characterization unit. Position the carrier this way to insure correct identification of the sample wells.

3. Load the DNA Prep Carrier

1. Remove the DNA Prep carrier from the refrigerator.

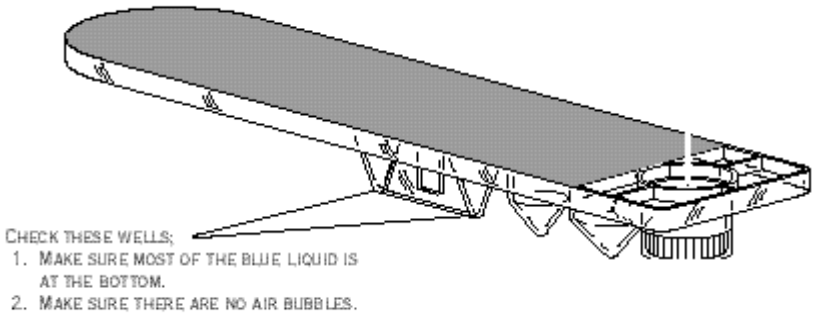
2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**

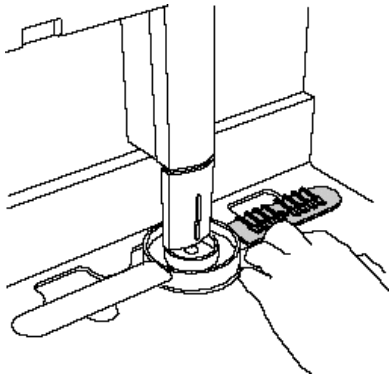
3. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.

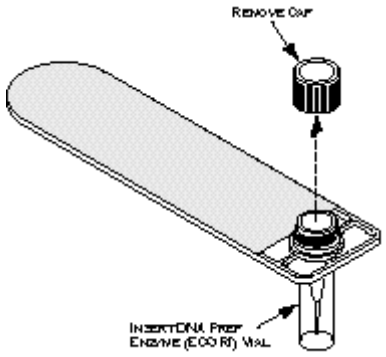
4. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as a 50 U/µL working stock as follows.**

   <u>50 U/µL:</u> 26.5 µL *Hin*d III and 26.5 µL of NEB 10X Buffer 2

   During addition of the Buffer, mix enzyme and buffer to homogeneity by stirring with the micropipette tip.





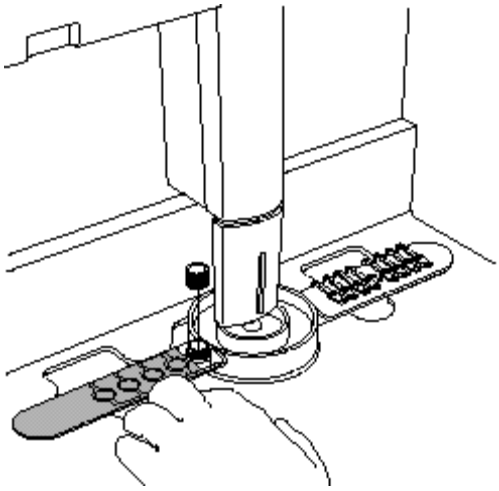


5. Remove the cap from the Enzyme vial.

6. Insert the vial into the carrier.

7. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.

8. Push the DNA Prep carrier down firmly until it snaps into place.

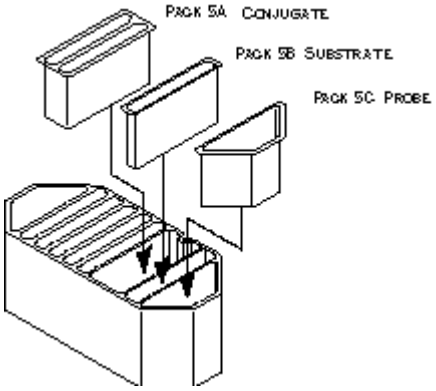


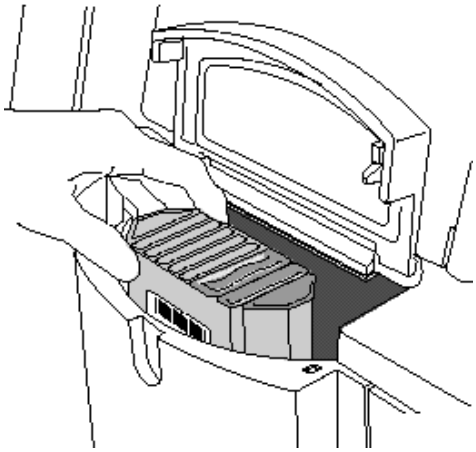
4. Load the MP Base and Carousel

1. Unpack the disposables.

2. Remove the MP base (Pack 5) from the incubator and the Conjugate (Pack 5A), Substrate (Pack 5B), and Probe (Pack 5C) from the refrigerator.

3. Remove each insert from its pouch. Tap the powdered reagent packs gently to bring all powder to the bottom of the packs. Place reagent packs in the MP base and load the base in the carousel.

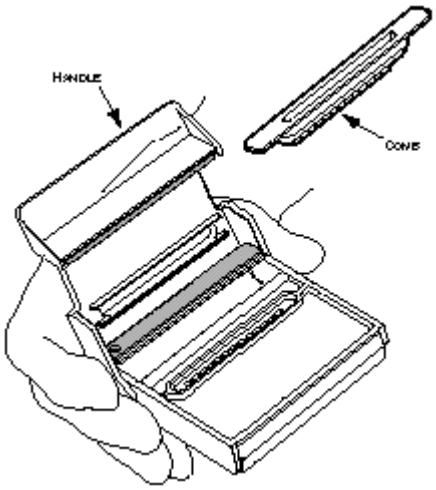


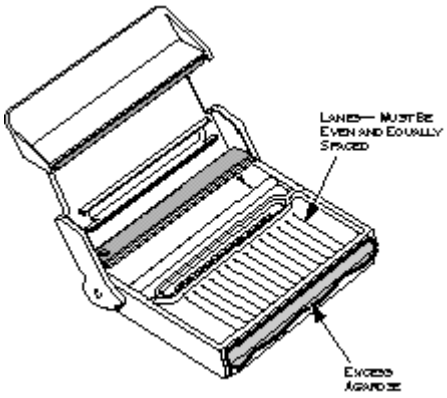


CAUTION! Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.

5. Load the Gel Cassette

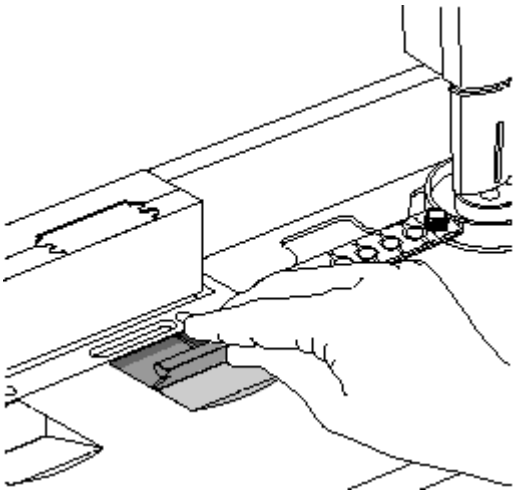
1. Remove the gel cassette from its package.
2. Grasp one end of the rubber comb and gently pull the comb from the cassette.
3. Unfold the handle of the cassette towards you until the handle snaps into place.
4. Check the front edge of the gel cassette and the lanes of the gel.





Warning! *If the cassette shows a build up of excess gel on the front edge, or if you notice any shrinkage of the gel away from the cassette or bubbles, record the lot number and call Customer Support. Use a new cassette for this run.*

5. Insert the gel cassette into the slot labeled **Gel Bay.** The RiboPrinter® system will prevent the insertion of the cassette into the incorrect slot by blocking one slot with the LDD Pipette.

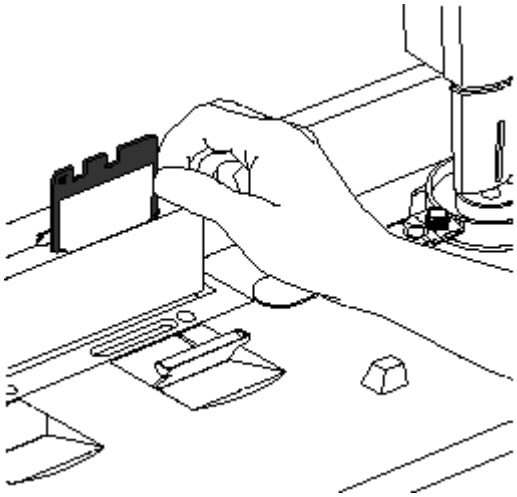


6. Press the cassette forward firmly until it snaps into place.

6. Load the Membrane

1. Grasp the membrane and carefully drop it into the front slot and flip the metal bracket against the back of the membrane.

CAUTION! You can insert the membrane backwards. This will cause an alarm that prevents the sample from being processed until the error is corrected. Always make certain that the two large slots are on top and that the square hole on the side faces your left as you insert the membrane.



7. Close all doors and the instrument will begin sample processing.

8. Load the Next Batch

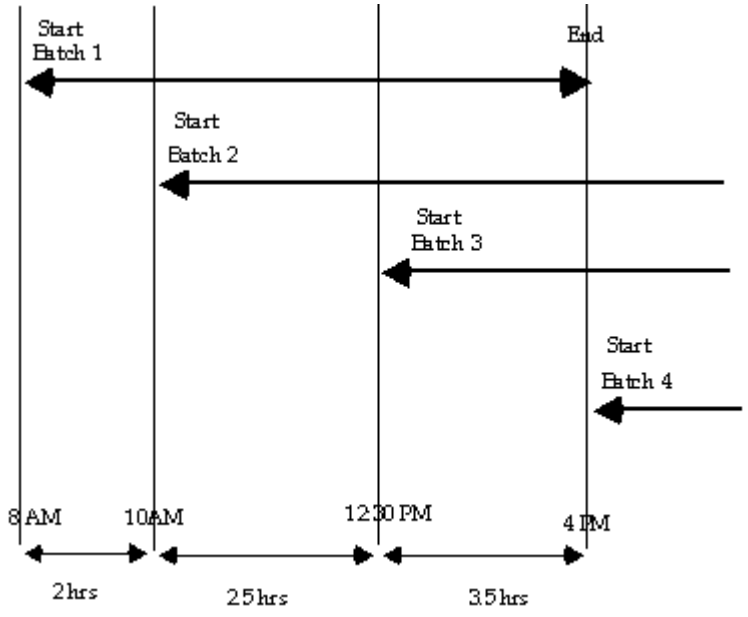
The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.
The chart above shows the approximate loading times for each batch in a work shift using only the VCA protocol.

1. You can now use the **Create Batch** option to set up a new pending batch.

2. When you complete the information window and click on the **Start Normal Batch** option, the window displays a message telling you when you can load the next batch.

Batch Report

After image processing is completed, the system automatically runs a series of analysis functions and generates a Batch Information Report. This task does not require any action on the part of the operator. Reports are automatically saved to the hard disk of the computer and sent to the printer.



Casarez, E. A.; Pillai, S. D.; Mott, J.; Vargas, M.; Dean, K.; Di Giovanni, G. D. (2007). "Direct comparison of four bacterial source tracking methods and a novel use of composite data sets." J. Appl. Microbiol. In press doi:10.1111/j.1365-2672.2006.03246.x.

This Page Left Blank Intentionally

E-4: *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. Once per quarter, samples should be shipped overnight on dry-ice to SAML. Dry-ice blocks should be packed on both top and bottom of the cooler for shipment. Extra care should be taken to ensure filters do not thaw in transport by not overcrowding the cooler and using adequate amounts of dry ice.

5. Notification of shipment should be sent to SAML via email, emartin@ag.tamu.edu, or phone, SAML Lab 979-845-5604, no later than the day of shipping. Notification should include tracking number and direct Ana-Lab contact person for confirmation upon receipt of samples.

6. 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 PCR Master Mix

1. Prepare sufficient PCR Master Mix for samples and controls, as well as one blank per 10 samples to account for volume loss due to repeat pipetting.

Bacteroidales **PCR Master Mix – per sample**

MASTER MIX	Amt (uL)	Final Calc	Final Units
Molecular Grade Water	**30.2**		
10X PCR buffer I w Mg (ABI)	**5**	1	X
MgCl2 (25 mM) (ABI)	**1**	0.5 (2.0 final)	mM
each dGTP, dCTP, dATP (33 mM mix) (Amersham)	**0.3**	200	uM each
dUTP (100 mM) (Amersham)	**0.2**	400	uM
Bacteroidales Primer Mix	**5**	200	nM each
BSA (30 mg/mL)	**2.5**	1.5	ug/uL
AmpliTaqGold (Units)	**0.5**	2.5	Units/rxn
Uracil DNA glycosylase NEB (UDG; 1 U/rxn)	**0.25**	0.5	Units/rxn

2. Dispense 45 µl of Master Mix for each sample into the appropriate well of PCR plate.

3. Briefly vortex DNA extracts, quick spin, then add 5 µl to the appropriate PCR well.

4. Carefully seal plate using an adhesive PCR cover.

5. Load the plate into the thermal cycler and run under the appropriate *Bacteroidales* program with the following cycling conditions:

    a. UDG digestion 50°C for 10 min

    b. Initial denaturation at 95°C for 10 min

    c. 40 Cycles:
        i. Denaturation at 95°C for 30 sec
        ii. Annealing at 53°C to 62°C (depending on primer set) for 1 min
        iii. Extension at 72°C for 1 min

    d. Final Extension at 72°C for 10 min

6. Store completed reactions at -20°C until analyzed by gel electrophoresis.

7. Prepare a 200 mL, 2% agarose gel using a 500 mL bottle. Add 200 mL of 1 X TBE buffer and 4.0 g agarose. Microwave until agarose is fully dissolved, add 10 µl of ethidium bromide (10 mg/ml), tighten cap, swirl to mix and let cool 1-2 minutes.

8. Pour agarose into casting tray with one or two 20-tooth, 0.75 mm thick combs.

9. Allow gel to solidify for 30-60 minutes on the bench, remove comb(s), and place in gel tank with TBE buffer. Discard TBE in gel tank after it has been used twice.

10. The following items will be needed for electrophoresis:

    <u>100 bp ladder (0.33 µg/10 µL) (1500 µL final, enough for 150 lanes)</u>

    200 µL Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µL 100 bp ladder (add reagents below to a full tube of marker)

    300 µL 6X Loading Buffer (see recipe below)

    150 µL 10X PCR buffer

    850 µL molecular grade water

    Store in cold room

    <u>6X Loading Buffer</u>

    25 mg bromphenol blue (0.25%)

    1.5 g ficoll 400 (15%)

    Add molecular grade water to 10 mL, divide into 1 mL aliquots and freeze, the aliquot currently being used can be stored in the cold room

11. Mix 10 µl of PCR product with 2 µl of 6X Loading Buffer in the appropriate well of a Nunc Module.

12. Load the gel, starting with 10 µl of 100 bp ladder in the first lane, followed by 12 µl of each sample with Loading Buffer, and 10 µl of 100 bp ladder after the last sample.

13. Start electrophoresis power supply set at 100 volts, run for 1.5 hours.

14. Follow Gel Imager SOP for image capture. Save digital photograph as an 8-bit TIFF file with no scaling and print a hardcopy for notebook.

This Page Left Blank Intentionally

APPENDIX F

Sample Handling and Shipping for EPA Method 1603

This Page Left Blank Intentionally

Shipping and Handling of modified mTEC plates from EPA Method 1603

1. After 22 +/- 2 hour incubation, red or magenta colonies are considered 'typical' *E. coli*.

2. Colonies counted should be indicated with a 'dot' on the back of the plate to ensure isolation of *E. coli* grown during the incubation period. Total number of counts should also be included on the back of each plate. In order to facilitate isolations, include at least one plate per sample having a countable number of *E. coli* colonies (20-80/plate).

3. Each plate should be sealed with parafilm around the edge to protect the filters from contamination. Dilution series for each sample should subsequently be grouped together either by parafilm or zip-top bag for transport.

4. The day following filtration, but no later than two days following filtration, plates should be shipped overnight to SAML at 4ºC. 'Blue-ice' or freezer blocks should be used to keep the samples cool, but not frozen in transport. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.

5. If sampling occurs over two days, the first day's plates should be counted 24 hours post filtration, sealed and placed 'media-side up' or 'upside down', so condensation does not fall onto the filter, and stored 4ºC until a complete sample set can be shipped together the next day.

6. Notification of shipment should be sent to SAML via email, emartin@ag.tamu.edu, or phone, SAML Lab 979-845-5604, no later than the day of overnight shipping. Notification should include *E. coli* count datasheet, tracking number, and direct SFA WET Lab contact person for confirmation upon receipt of samples.

This Page Left Blank Intentionally

APPENDIX G

Data Review Checklist
and
Data Summary Sheet

This Page Left Blank Intentionally

Data Review Checklist

Title of associated QAPP: _____

√, X, or N/A

Data Format and Structure

A. Are there any duplicate *Tag ID* numbers? _____
B. Are the *Tag prefixes* correct? _____
C. Are all *Tag ID* numbers 7 characters? _____
D. Are TCEQ station location (SLOC) numbers assigned? _____
E. Are sampling *Dates* in the correct format, MM/DD/YYYY? _____
F. Is the sampling *Time* based on the 24-hour clock (e.g. 13:04)? _____
G. Is the *Comment* field filled in where appropriate (e.g. unusual occurrence, sampling problems, unrepresentative of ambient water quality) and any punctuation deleted?

_____

H. *Source Code 1, 2* and *Program Code* are valid and used correctly? _____
I. Is the sampling date in the *Results* file the same as the one in the *Events* file? _____
J. Values represented by a valid parameter (*STORET*) code with the correct units and leading zeros? _____
K. Are there any duplicate parameter codes for the same *Tag Id*? _____
L. Are there any invalid symbols in the Greater Than/Less Than (*GT/LT*) field? _____
M. Are there any tag numbers in the *Results* file that are not in the *Events* file? _____
N. Have confirmed outliers been identified? (with a ■1" in the Verify_flg field) _____
O. Have grab data (bacteria, for example) taken during 24-hr events been reported separately as RT samples? _____
P. Is the file in the correct format (ASCII pipe-delimited text)? _____

Data Quality Review

A. Are all the values reported at or below the AWRL? _____
B. Have the outliers been verified? _____
C. Checks on correctness of analysis or data reasonableness performed?
e.g.: Is ortho-phosphorus less than total phosphorus?
Are dissolved metal concentrations less than or equal to total metals? _____
D. Have at least 10% of the data in the data set been reviewed against the field and laboratory data sheets? _____
E. Are all parameter codes in the data set listed in the QAPP? _____
F. Are all stations in the data set listed in the QAPP? _____

Documentation Review

A. Are blank results acceptable as specified in the QAPP? _____
B. Were control charts used to determine the acceptability of field duplicates? _____
C. Was documentation of any unusual occurrences that may affect water quality included in the Event file Comments field? _____
D. Were there any failures in sampling methods and/or deviations from sample design requirements that resulted in unreportable data? If yes, explain on next page. _____
E. Were there any failures in field and laboratory measurement systems that were not resolvable and resulted in unreportable data? If yes, explain on next page. _____

√ = Yes **X = No** **N/A** = Not applicable

Describe any data reporting inconsistencies with AWRL specifications. Explain failures in sampling methods and field and laboratory measurement systems that resulted in data that could not be reported to the TCEQ. (attach another page if necessary):

_____

_____

_____

_____

_____

Date Submitted to TCEQ: _____
Tag ID Series: _____
Date Range: _____
Data Source: _____
Comments (attach README.TXT file if applicable):

_____

_____

_____

_____

Planning Agency's Data Manager Signature: _____

Date: _____

DATA SUMMARY

Data Set Information

Data Source: _____.

Date Submitted: _____.

Tag_id Range: _____.

Date Range: _____.

Comments:

Please explain in the space below any data discrepancies discovered during data review including:

- Inconsistencies with AWRL specifications or LOQs
- Failures in sampling methods and/or laboratory procedures that resulted in data that could not be reported to the TCEQ (indicate items for which the Corrective Action Process has been initiated).
- Include completed Corrective Action Plans with the applicable Progress Report.

_____

_____

_____

_____

_____

_____

_____

_____

☐ I certify that all data in this data set meets the requirements specified in Texas Water Code Chapter 5, Subchapter R (TWC §5.801 et seq) and Title 30 Texas Administrative Code Chapter 25, Subchapters A & B.

☐ This data set has been reviewed using the Data Review Checklist.

Planning Agency Data Manager: _____.

Date: _____.

This Page Left Blank Intentionally

APPENDIX H

RUAA DOCUMENTATION

Includes:

Contact Information Form

Field Data Sheets – Basic RUAA Survey

Comprehensive RUAA Interview Form

RUAA Summary

This Page Left Blank Intentionally

Contact Information Form

(This form must be completed prior to conducting a Basic RUAA Survey and/or Comprehensive RUAA.)

The TCEQ Water Quality Standards Group will not consider or review a RUAA unless the appropriate entities listed below have been notified prior to the beginning of a RUAA. A RUAA should not be conducted until you have received a Notice to Proceed from the TCEQ Water Quality Standards Group.

River or stream name: _____

Required Local Contacts:

Ask the contacts if a recreational use-attainability analysis is appropriate for the river or stream and check Yes or No below. Document the name of the person contacted and the date they were notified about the proposed RUAA project.

Clean Rivers Program Partner (River Authority and other local partners)	☐ Yes ☐ No Date Notified: _____ Name: _____
Texas Parks and Wildlife Department region staff	☐ Yes ☐ No Date Notified: _____ Name: _____
TCEQ region staff	☐ Yes ☐ No Date Notified: _____ Name: _____
Texas State Soil and Water Conservation Board Statewide Resource Management Group srm-team@tsswcb.state.tx.us	☐ Yes ☐ No Date Notified: _____ Name: _____

Suggested Additional Local Contacts:

If contacted, ask the contacts if a recreational use-attainability analysis is appropriate for the river or stream and check Yes or No below If contacted, include information regarding notification date and person contacted on a separate page and attach it to this form.

Local Parks and Recreation Departments Project Area	☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in
Municipal Government/Jurisdiction Project Area	☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in
County Government/Jurisdiction	☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in
Project Area Local Recreation Groups	☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in
Project Area Conservation Groups Project Area	☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in

Local Soil and Water Conservation Districts ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Texas AgriLife Extension Service ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area (local County Extension Agent)

USDA Natural Resources Conservation Service ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area (local field staff)

Watershed Groups ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Long-term Landowners/Adjacent Landowners ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Texas Stream Team (formerly Texas Watch) ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Canoe Clubs ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

City Commissioners Office ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Real estate agents ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Local non-profits ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

City/county offices ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area
(Engineer, Health, Law Enforcement)

Flood control districts ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Councils of Governments ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Texas Parks and Wildlife Department Game Warden ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Other:_____ ☐ Yes ☐ No ☐ Entity Not Contacted ☐ Entity Not in Project Area

Draft Definitions (2010 TSWQS Revision)

- Primary contact recreation: Water recreation activities, such as wading by children, swimming, water skiing, diving, tubing, surfing, and whitewater kayaking, canoeing, and rafting, involving a significant risk of ingestion of water.
- Secondary contact recreation 1: Water recreation activities, such as fishing, commercial and recreational boating, and limited body contact incidental to shoreline activity, not involving a significant risk of water ingestion and that commonly occur.
- Secondary contact recreation 2: Water recreation activities, such as fishing, commercial and recreational boating, and limited body contact incidental to shoreline activity, not involving a significant risk of water ingestion but that occur less frequently than for secondary contact recreation 1 due to (1) physical characteristics of the waterbody and/or (2) limited public access.
- Noncontact recreation: Activities, such as ship and barge traffic, birding, and using hike and bike trails near a waterbody, not involving a significant risk of water ingestion, and where primary and secondary contact recreation should not occur because of unsafe conditions.

Information from Local Contacts:

1. If any entity answered no, please list the reason(s) why:

2. Did the local entities confirm that primary contact recreation activities frequently occur? ☐ Yes ☐ No
Please describe how often the activities occur? ☐ Unknown ☐ Never ☐ Daily ☐ Monthly ☐ Yearly
If no, explain:

3. Did the local entities confirm that secondary contact recreation 1 activities frequently occur? ☐ Yes ☐ No
Please describe how often the activities occur? ☐ Unknown ☐ Never ☐ Daily ☐ Monthly ☐ Yearly
If no, explain:

4. Did the local entities confirm that secondary contact recreation 2 activities frequently occur? ☐ Yes ☐ No
Please describe how often the activities occur? ☐ Unknown ☐ Never ☐ Daily ☐ Monthly ☐ Yearly
If no, explain:

5. Did the local entities confirm that noncontact recreation activities frequently occur? ☐ Yes ☐ No
Please describe how often the activities occur? ☐ Unknown ☐ Never ☐ Daily ☐ Monthly ☐ Yearly
If no, explain:

6. Do the local entities know if this waterbody provides substantial flow to a waterbody with primary contact recreation activities (e.g., swimming in a state/local park) or a bathing beach that is located immediately downstream? ☐ Yes ☐ No ☐ Unknown

If yes, have the local entities provide the name of the waterbody and a description of the location of the primary contact recreation uses or bathing beach.

<u>Notify TCEQ Water Quality Standards Group (required):</u>

Send an e-mail notification to the TCEQ Water Quality Standards Group at standards@tceq.state.tx.us.
Notified: ☐ Yes ☐ No
Date Notified by e-mail: _____
Date TCEQ WQS e-mail Response Received: ____
WQS Group Contact Person Providing Response: _____
Did the WQS Group provide a Notice to Proceed with the RUAA? ☐ Yes ☐ No

Additional Local Contacts Made:

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Name: _____
Entity: _____

(to be completed for each site)

Data Collectors & Contact Information:
Date & Time: County Name:
Stream Name:
Segment No. or nearest downstream Segment No.:
Description of Site:

A. Stream Characteristics:

1. Check the following channel flow status that applies.
   ☐ dry ☐no flow ☐low ☐normal ☐high ☐flooded

2. Check the following stream type that applies on the day of the survey:
   - ☐ Ephemeral: A stream which flows only during or immediately after a rainfall event, and contains no refuge pools capable of sustaining a viable community of aquatic organisms.
   - ☐ Intermittent: A stream which has a period of zero flow for at least one week during most years. Where flow records are available, a stream with a 7Q2 flow of less than 0.1 cubic feet per second is considered intermittent.
   - ☐ Intermittent w/ perennial pools: An intermittent stream which maintains persistent pools even when flow in the stream is less than 0.1 cubic feet per second.
   - ☐ Perennial: A stream which flows continuously throughout the year. Perennial streams have a 7Q2 equal to or greater than 0.1 cubic feet per second.
   - ☐ Designated or unclassified tidal stream: A stream that is tidally influenced. If you checked this box, you will need to contact the Water Quality Standards Group and evaluate whether or not a bathing beach is located along the tidal stream and whether or not a bathing beach is located along the estuary, bay or Gulf water that the tidal stream flows into.

3. Streamflow
   Use USGS gage data (if a gage is located at a site or within a quarter mile of a site) or use the Stream Flow (Discharge) Measurement Form and follow the procedures outlined in the most recent TCEQ Surface Water Quality Monitoring Procedures, Volume 1, RG-415. If USGS gage data is used for a site, include that information as an attachment and list the streamflow on the sampling date below. If the stream flow taken at one site is representative of the flow at another site(s), then that flow can be used as the observed flow and should be documented below. If the stream flow measured at one site is different from another site, then stream flow should be taken at both sites.
   _____ cfs

4. Water Quality Data (Field Parameters)
   Field parameters should be collected in accordance with the procedures outlined in the most recent TCEQ Surface Water Quality Monitoring Procedures, Volume 1.

Air Temp: _____º C Water Temp: _____º C

5. Riparian Zone (Mark dominant categories with L (Left Bank) and R (Right Bank). Bank orientation is determined by the investigator facing downstream.)
   _____ Forest _____ Urban _____ Rip rap
   _____ Shrub dominated corridor _____ Pasture _____ Concrete
   _____ Herbaceous marsh _____ Row crops Other (specify): _____
   _____ Mowed/maintained corridor _____ Denuded/Eroded bank

6. Ease of bank access to the water body: ☐ Easy ☐ Moderately difficult ☐ Difficult

7. Please describe access opportunities or explain why the site is not easily accessible (Attach photos for documentation):
   _____
   _____
   _____

8. Dominant Primary Substrate
   ☐Cobble ☐Sand ☐Silt ☐Mud/Clay ☐Gravel ☐Bedrock ☐Rip rap ☐ Concrete

Field Data Sheets – Basic RUAA Survey

Stream Name: _____ Site: _____
Date: _____ Time: _____

B. Primary Contact Water Recreation Evaluation:

- Primary contact recreation draft definition: Water recreation activities, such as wading by children, swimming, water skiing, diving, tubing, surfing, and whitewater kayaking, canoeing, and rafting, involving a significant risk of ingestion of water.

1. Were water recreation activities that involve a significant risk of ingestion (full body immersion) observed at this site? ☐ Yes ☐ No primary contact recreation activities were observed

   a. <u>Check the following boxes of primary contact recreation activities observed at the time of the sampling event at the site (Attach photos of the activities or lack of activities).</u>
      ☐ Wading-Children ☐ Tubing ☐ No primary contact activities that commonly occur were observed
      ☐ Wading-Adults ☐ Surfing ☐ Swimming ☐ Whitewater-kayaking, canoeing, rafting
      ☐ Water skiing ☐ Diving ☐ Other: _____

□ frequent public swimming-created by publicly owned land or commercial operations

b. Check the number of individuals observed at the site: □ None □ 1-10 □ 11-20 □ 20-50 □ >50

c. Check the following that apply regarding the individuals proximity to the water body.
□ Water in mouth or nose of the individual
□ Primary touch: Individual's body (or portion) immersed in water
□ Secondary touch: fishing, pets and related contact with water
□ Individual is in a boat touching water
□ Individual is on shore near water within 8 meters (25ft) of water
□ Individual is well away from water between 8 and 30 meters (100 ft) □ Not applicable

2. If primary contact recreation activities are not observed, describe the physical characteristics of the water body that may hinder the frequency of primary contact (depth, etc.) (Attach photos, etc. for documentation).
_____
_____
_____

3. Describe if there is public access (e.g., parks, roads, etc.) (Attach photos, maps, etc. for documentation).
_____
_____
_____

4. Is an area with primary contact recreation activities or a bathing beach (e.g., state/local parks with swimming, etc.) located near (e.g., within 5 miles upstream and downstream) this site?

C. Secondary Contact Water Recreation Evaluation:

- Secondary contact recreation 1: Water recreation activities, such as fishing, commercial and recreational boating, and limited body contact incidental to shoreline activity, not involving a significant risk of water ingestion and that commonly occur.
- Secondary contact recreation 2: Water recreation activities, such as fishing, commercial and recreational boating, and limited body contact incidental to shoreline activity, not involving a significant risk of water ingestion but that occur less frequently than for secondary contact recreation 1 due to (1) physical characteristics of the water body and/or (2) limited public access.

1. Were water recreation activities observed at the site, but the nature of the recreation does not involve a significant risk of ingestion (e.g., secondary contact recreation activities)? □ Yes □ No secondary contact recreation activities were observed.

   a. Check the following boxes of secondary contact recreation activities that were observed at the time of the sampling event at the site (Attach photos of activities or lack of activities).
      □ Fishing
      □ Boating-commercial, recreational
      □ Non-whitewater-kayaking, rafting, canoeing
      □ No secondary contact recreation activities were observed
      □ Other secondary contact activities: _____

Field Data Sheets – Basic RUAA Survey

Stream Name: _____ Site: _____
Date: _____ Time: _____

   b. Check the number of individuals observed at the site.
      □ None □ 1-10 □ 11-20 □ 20-50 □ greater than 50

   c. Check the following that apply regarding the individuals proximity to the water body.
      □ Secondary touch: fishing, pets and related contact with water
      □ In a boat touching water
      □ Body on shore near water within 8 meters (25ft) of water
      □ Body well away from water between 8 and 30 meters (100 ft)

2. If secondary contact recreation activities are not observed, describe the physical characteristics of the water body that may hinder the frequency of secondary contact (Attach photos, etc. for documentation).
   _____
   _____
   _____

3. If secondary contact recreation activities are observed, how often do water recreational activities occur that do not involve a significant risk of water ingestion?
   ☐ frequently ☐infrequently
   Please describe how often the activities occur? □ Unknown □ Never □ Daily □ Monthly □ Yearl

4. If infrequently, what is the reason?
   ☐ physical characteristics of the water body ☐limited public access ☐other
   If other, list reasons:
   _____
   ____

5. Describe the physical characteristics of the water body that hinders the frequency of secondary contact recreation (depth, etc.) (Attach photos or depth measurements, etc. for documentation).

_____
_____
_____
_____

6. Describe why there is limited public access (e.g., lack of roads, river or stream banks overgrown, etc.) (Attach photos, maps, etc. for documentation).

_____
_____
_____
_____

D. Noncontact Recreation Evaluation

Noncontact recreation applies to water bodies where recreation activities do not involve a significant risk of water ingestion, and where primary and secondary contact recreation uses do not occur because of unsafe conditions, such as barge traffic.

1. Provide site-specific information and documentation (including photographs) regarding unsafe conditions, recreation activities, and presence or absence of water recreation activities.

_____
_____
_____
_____
_____
_____
_____
_____

Field Data Sheets – Basic RUAA Survey

Stream Name: _____ Site: _____
Date: _____ Time: _____

E. Stream Channel and Substantial Pools Measurements

Please check the following which best describes the river or stream: □ Wadeable □ Non-wadeable

1. Wadeable Streams

Determine whether or not the average depth at the thalweg is greater than 0.5 meters and if there are substantial pools with a depth of 1 meter or greater. Walk an approximately 300 meter reach (total) at the site and take the following measurements within the 300 meter reach. Measurements should be taken during base flow conditions (sustained or typical dry, warm-weather flows between rainfall events, excluding unusual antecedent conditions of drought or wet weather

Also, take photos facing upstream, downstream, left bank, and right bank at the 30 meters, 150 meters, and 300 meters.

Photos #s (30 meters) Upstream____ Downstream____ Left Bank____ Right Bank____

Photos #s (150 meters) Upstream____ Downstream____ Left Bank____ Right Bank____

Photos #s (300 meters) Upstream____ Downstream____ Left Bank____ Right Bank____

a) Substantial pools - Measure the length of each pool (if > 10 pools only measure 10 pools), the width (at the widest point), and the deepest depth. A substantial pool is considered a pool greater than 10 meters in length for the purposes of a Basic RUAA Survey. If depth and/or width measurements were not attainable, explain why.

	Length (meters)	Width (meters)	Depth (meters)
Pool 1			
Pool 2			
Pool 3			
Pool 4			
Pool 5			
Pool 6			
Pool 7			
Pool 8			
Pool 9			
Pool 10			

b) Average depth at the thalweg –Take depth measurements approximately every 30 meters to calculate an average depth at the thalweg (at least 10 measurements needed). If depth and/or width measurements were not attainable, explain why.

Distance	Depth (meters)
30 meters	
60 meters	
90 meters	
120 meters	
150 meters	
180 meters	
210 meters	
240 meters	
270 meters	
300 meters	
Average	

Field Data Sheets – Basic RUAA Survey

Stream Name: _____ Site: _____
Date: _____ Time: _____

c) Stream width – Measure (1) the width at one point which represents the typical average width of the 300 meter reach; (2) the width at the narrowest point of the stream within the 300 meter reach; and (3) the width at the widest point of the stream within the 300 meter reach.

Measurement Type	Width (meters)
Typical Average Width of 300 meter reach	
Width at narrowest point of the stream within 300 meter reach	
Width at the widest point of the stream within 300 meter reach	

d) Is there sufficient water within a 300 meter stream reach during base flow conditions to support primary contact recreation? □ Yes □ No
Comments:_____
_____
_____

2. Non-wadeable Streams
If accessible, take 10 width measurements which represent typical widths of the 300 meter reach. If the water is too deep and not accessible record the estimated average width of the water body.

Also, take photos facing upstream, downstream, left bank, and right bank at .

Photos #s (30 meters) Upstream____ Downstream____ Left Bank____ Right Bank____
Photos #s (150 meters) Upstream____ Downstream____ Left Bank____ Right Bank____
Photos #s (300 meters) Upstream____ Downstream____ Left Bank____ Right Bank____

# Measurements	Width (meters)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

Field Data Sheets – Basic RUAA Survey

Stream Name: _____ Site: _____
Date: _____ Time: _____

F. Additional RUAA Information

1. Check the following activities observed over the site reach.
   - ☐ Drinking or water in mouth
   - ☐ Playing on shoreline
   - ☐ Bathing
   - ☐ Picnicking
   - ☐ Walking
   - ☐ Motorcycle/ATV
   - ☐ Jogging/running
   - ☐ Hunting/Trapping
   - ☐ Bicycling
   - ☐ Wildlife watching
   - ☐ Standing
   - ☐ None
   - ☐ Sitting
   - ☐ Other: _____
   - ☐ Lying down/sleeping

2. Are there permanent or long-term hydrologic modifications that are constructed and operated in a way that affects the recreational uses? ☐ Yes ☐ No (If yes, please provide supporting documentation and photos.)
   Comments:_____
   _____
   _____

3. Check any channel obstructions that apply (Attach photos).

☐ Culverts ☐ Fences ☐ Log jams ☐ Rip rap ☐ Water control structure
☐ Barbed wire ☐ Dams ☐ Thick vegetation ☐ Low bridges ☐ None
☐ Utility pipe ☐ Other (specify):_____

4. Check all surrounding conditions that promote recreational activities (Attach photos of evidence or unusual items of interest).

☐ Campgrounds ☐ Stairs/walkway ☐ Roads (paved/unpaved) ☐ Other:_____
☐ Playgrounds ☐ Boating access (ramps) ☐ Populated area ☐ None of the Above
☐ Rural area ☐ Beach ☐ Docks or rafts
☐ Residential ☐ Bridge crossing ☐ Commercial outfitter
☐ National forests ☐ Commercial boating ☐ Nearby school
☐ Urban/suburban location ☐ Trails/paths (hiking/biking) ☐ Power Line Corridor
☐ Golf Course ☐ Paved parking lot ☐ Parks (national/city/county/state)
☐ Sports Field ☐ Unimproved parking lot ☐ Public Property

Comments:_____
_____
_____

5. Check all surrounding conditions that impede recreational activities (Attach photos of evidence or unusual items of interest).

☐ Private Property ☐ Fence ☐ No trespass sign
☐ Barge/ship traffic ☐ Wildlife ☐ Industrial
☐ Steep slopes ☐ None of the Above ☐ No public access
☐ Other:_____ ☐ No roads

Comments:_____
_____
_____

6. Check any indications of human use (Attach photos).

☐ Roads ☐ RV/ATV Tracks ☐ NPDES Discharge ☐ Organized event
☐ Rope swings ☐ Camping Sites ☐ Gates on corridor ☐ No Human Presence
☐ Dock/platform ☐ Fire pit/ring ☐ Children's toys
☐ Foot paths/prints ☐ Fishing Tackle ☐ Remnant's of Kid's play
☐ Other: _____

Comments:_____
_____
_____

Field Data Sheets – Basic RUAA Survey

Stream Name: _____ Site: _____
Date: _____ Time: _____

7. Check all water characteristics that apply (Attach photos).
   Aquatic Vegetation: □ absent □ rare □common □abundant
   Algae Cover: □ absent □ rare □common □abundant
   Odor: □ none □ rare □common □abundant
   Color: □ clear □ green □red □ brown □ black
   Bottom Deposit: □ sludge □ solids □ fine sediments □none □other
   Water Surface: □ clear □ scum □ foam □ debris □ oil
   Other:_____
   _____
   _____

8. Vertebrates Observed within 300 meter reach
   Snakes ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Water Dependent Birds ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Alligators ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Comments:_____
   _____

9. Mammals Observed within 300 meter reach
   Wild ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Domesticated Pets ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Livestock ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Feral Hogs ☐ None ☐ slight presence ☐ moderate presence ☐ large presence
   Comments:_____
   _____

10. Evidence of wild animals or evidence of birds, cattle, hogs, etc.
    ☐ Tracks ☐ Fecal droppings ☐ Bird nests

11. Garbage Observed
    Large garbage in the channel ☐ None ☐ Rare ☐ Common ☐ Abundant
    Small garbage in the channel ☐ None ☐ Rare ☐ Common ☐ Abundant
    Bank Garbage ☐ None ☐ Rare ☐ Common ☐ Abundant

Briefly describe the kinds of garbage observed:

_____
_____
_____

12. Is the site located in a wildlife preserve with large wildlife (i.e., waterfowl) population? ☐ Yes ☐ No

13. Please document any other relevant information regarding recreational activities and the water body in general (for example, area outside of the stream reach evaluated).

_____
_____
_____
_____
_____
_____
_____

Field Data Sheet - Basic RUAA Survey

Stream Flow (Discharge) Measurement

Stream:_____ Date:_____

Site:_____ Site Description:_____

Time Begin:_____ Time End:_____ Meter Type:_____

Observers:_____ Stream Width*:_____ Section Width (W):_____

Observations:_____

Section Midpoint (ft) (m)	Section Depth (ft) (m) (cm) (D)	Observational Depth** (ft) (m)	Velocity (V)		Flow (Q) (m^3/s) (ft^3/s) Q = (W)(D)(V)
			At Point (ft/s)(m/s)	Average (ft/s)(m/s)	

Comprehensive RUAA Interview Form

Stream Name: _____ Segment #: _____ Site: _____

Interviewer's Name: _____

Date & Time (include AM or PM): _____

Interviewed: ☐ In person ☐ By phone ☐ By mail

☐ No interviews were conducted
If no interviews were conducted, please provide an explanation:
_____
_____
_____

*Are you willing to respond to a short survey about this stream? ☐ Yes ☐ No
If yes, complete contact information for the interviewee below. Do not collect name or contact information if interviewee is a minor. The contact information portion is not required if the interviewee does not want to provide this information.

Legal name: _____ Daytime phone number: _____
Mailing address: _____
Interviewee selected because (e.g., house adjacent to stream; standing by stream, etc.)
_____
_____
_____

Questions:

1. Are you familiar with this stream? ☐ Yes ☐ No
   _____
   If yes, proceed to #2. If no, stop here and do not conduct an interview.

2. Describe the location(s) of the stream reach the interviewee is familiar with:
   _____
   _____
   _____

3. Have the interviewer characterize the stream flow. Since the interviewer may not be familiar with TCEQ's definitions or distinction between the different water bodies, please refer to the definitions listed below when asking this question.

   ☐ Ephemeral: A stream which flows only during or immediately after a rainfall event, and contains no refuge pools capable of sustaining a viable community of aquatic organisms.

☐ Intermittent: A stream which has a period of zero flow for at least one week during most years. Where flow records are available, a stream with a 7Q2 flow of less than 0.1 cubic feet per second is considered intermittent. (Channel contains flowing water for only a portion of the year and surface water may be absent at times.)

☐ Intermittent w/ perennial pools: An intermittent stream which maintains persistent pools even when flow in the stream is less than 0.1 cubic feet per second. (When not flowing, the water may remain in isolated pools.)

☐ Perennial: A stream which flows continuously throughout the year. Perennial streams have 7Q2equal to or greater than 0.1 cubic feet per second.

4. Have you or your family personally used the stream for recreation? ☐ Yes ☐ No
   If yes, proceed to #6. If no, proceed to #5.

5. a. List reasons stream not used.

   _____

   _____

   _____

   b. Proceed to #7.

Comprehensive RUAA Interview Form

Stream Name: _____ Segment #: _____ Site: _____

6. How do you use the stream? When did these uses occur (e.g., year(s); season) and how often (times/year)? What location did these uses occur (get specific location and mark on a map)?
   ☐ Swimming ☐ Skin Diving ☐ Water Skiing ☐ Wind surfing ☐ Hunting ☐ Wading-Adults
   ☐ Tubing ☐ Kayaking ☐ Rafting ☐ Trapping ☐ SCUBA diving
   ☐ Snorkeling ☐ Fishing ☐ Boating ☐ Canoeing ☐ Wading-Children
   _____
   _____
   _____

7. Have you observed others using this stream for recreation? ☐ Yes ☐ No
   If yes, proceed to #8. If no, proceed to #9.

8. What kinds of uses have you witnessed? When did you witness these uses occurring (e.g., year(s); season) and how often (times/year)? What location did these uses occur (get specific location and mark on a map)?
   ☐ Swimming ☐ Skin Diving ☐ Water Skiing ☐ Wind surfing ☐ Hunting ☐ Wading-Adults
   ☐ Tubing ☐ Kayaking ☐ Rafting ☐ Trapping ☐ SCUBA diving
   ☐ Snorkeling ☐ Fishing ☐ Boating ☐ Canoeing ☐ Wading-Children
   _____
   _____
   _____

9. Have you heard about anyone using this stream for recreation? ☐ Yes ☐ No
   If yes, proceed to #10. If no, conclude the interview.

10. What kind of uses have you heard about? When did you hear that these uses occur (e.g., year(s); season) and how often (times/year)? What location did these uses occur (get specific location and mark on a map)?
    ☐ Swimming ☐ Skin Diving ☐ Water Skiing ☐ Wind surfing ☐ Hunting ☐ Wading-Adults
    ☐ Tubing ☐ Kayaking ☐ Rafting ☐ Trapping ☐ SCUBA diving
    ☐ Snorkeling ☐ Fishing ☐ Boating ☐ Canoeing ☐ Wading-Children

_____
_____
_____

11. Can you recommend someone else we could contact that knows the stream? ☐ Yes ☐ No
    If yes, list person's contact information:
    _____
    _____
    _____

12. Additional comments (from the interviewee or interviewer):
    _____
    _____
    _____
    _____

RUAA Summary
(Not part of the Field Data Sheet)

This form should be filled out after RUAA data collection is completed. Use the Contact Information Form, Field Data Sheets from all sites, Interview Forms from all interviews conducted, Historical Information Review, and other relevant information to answer the following questions on the waterbody.

Name of waterbody: _____
Segment # or Nearest Downstream Segment #: _____
Classified Segment?: _____
County: _____

1. Observations on Use
   a. Do primary contact recreation activities occur on the waterbody?
      ☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

   b. Do secondary contact recreation 1 activities occur on the waterbody?
      ☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

   c. Do secondary contact recreation 2 activities occur on the waterbody?
      ☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

   d. Do noncontact recreation activities occur on the waterbody?
      ☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

2. Physical Characteristics of waterbody
   a. What is the average thalweg depth? _____ meters

   b. Are there substantial pools deeper than 1 meter? ☐ yes ☐ no

   c. What is the general level of public access?
      ☐ easy ☐ moderate ☐ very limited

3. Hydrological Conditions (Based on Palmer Drought Severity Index)
   ☐ Mild-Extreme Drought ☐ Incipient dry spell ☐ Near Normal
   ☐ Incipient wet spell ☐ Mild-Extreme Wet

This Page Left Blank Intentionally

APPENDIX I

Attoyac Bayou Watershed
RUAA Field Survey Sites Distribution

RUAA Field Survey Site Map

This Page Left Blank Intentionally

Attoyac Bayou Watershed
RUAA Filed Survey Site Distribution

Proposed survey stations were selected in areas where the waterbodies are accessible to the public and have the highest potential for recreational use, such as identified public road crossings. Other than road crossings there are no other publicly accessible areas (parklands operated by public or private entities) with potential for recreational use directly along the six waterbodies of interest.

Attoyac Bayou

Approximate Length = 88.2 miles
Number of Stations = 18
Average miles between Stations = 4.4

- There is a station at each of the 13 identified road crossings and 5 private property locations
- There are 4 TPDES outfalls in this watershed; none are on the main stem of the Attoyac Bayou
    - 3 are WWTFs that are permitted to discharge treated wastewater at average daily flows not to exceed 120,000, 10,000, and 8,000 gpd respectively
    - 1 is a potable water treatment facility that is permitted to discharge filter backwash effluent at average daily flows not to exceed 200,000 gpd
- Ten additional private landowners have been contacted seeking their permission to allow site assessments on their property. To date, none of these have allowed site access.

Attoyac Bayou Tributaries

Big Iron Ore Creek

Approximate Length = 22.2 miles
Number of Stations = 5
Average miles between Stations = 3.12

- There is a station at each of the 5 identified road crossings and 0 private property locations
- Seven private landowners have been contacted seeking their permission to allow site assessments on their property. To date, none of these have agreed to allow site access.

Waffelow Creek

Approximate Length = 12.0 miles
Number of Stations = 3
Average miles between Stations = 2.5

- There is a station at each of the 3 identified road crossings and 0 private property locations
- Three private landowners have been contacted seeking their permission to allow site assessments on their property. To date, none of these have agreed to allow site access.

Terrapin Creek

Approximate Length = 9.4 mi
Number of Stations = 3
Average miles between Stations = 1.8

- There is a station at each of the 2 identified road crossings and 1 private property locations
- There is 1 TPDES outfall in this subwateshed; it is a WWTF permitted to discharge up to 8,000 gpd of treated effluent to a tributary of Terrapin Creek
- Two additional private landowners have been contacted seeking their permission to allow site assessments on their property. To date, none of these have agreed to allow site access.

West Creek

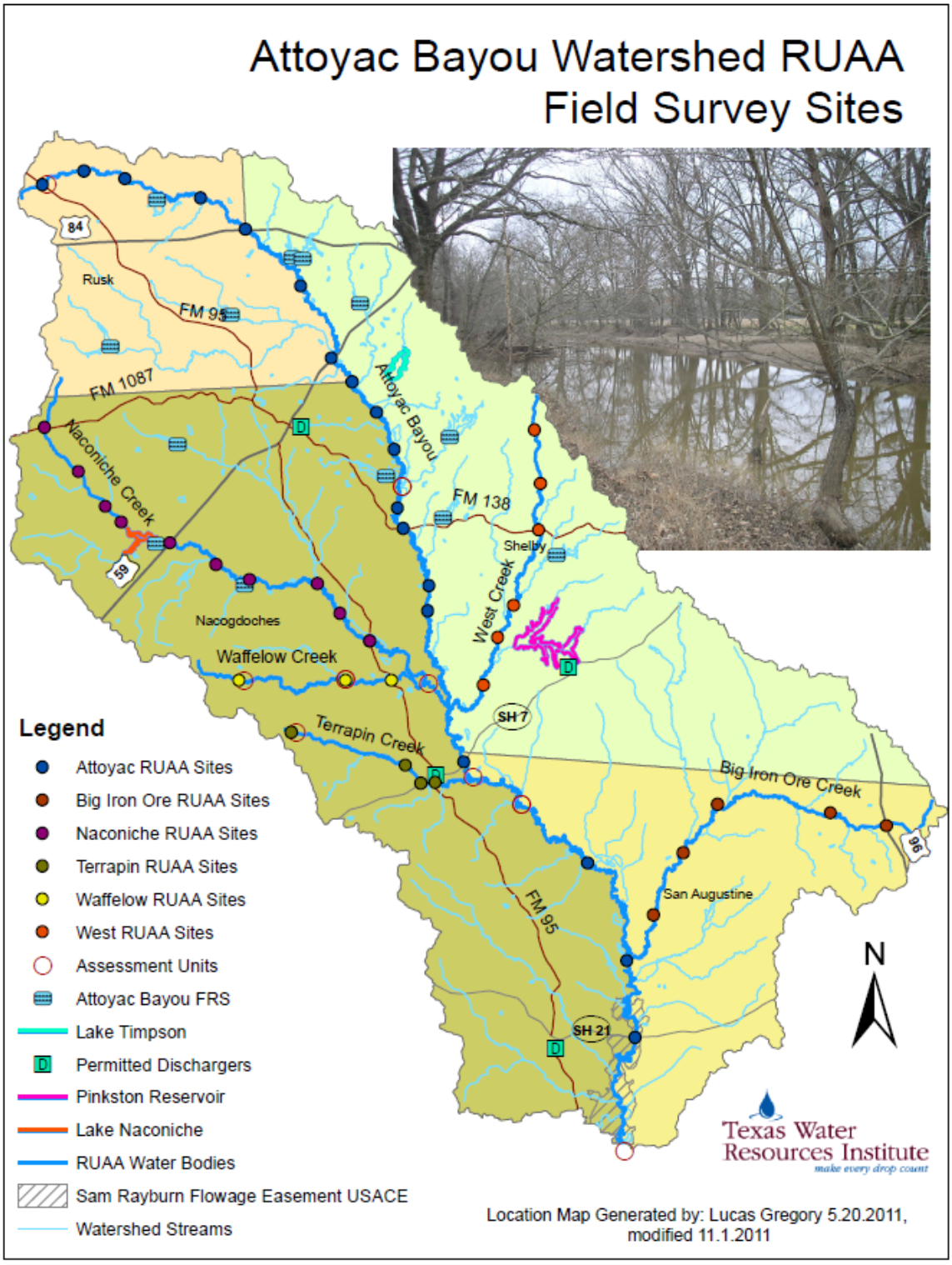
Approximate Length = 18.0 miles
Number of Stations = 6
Average miles between Stations = 2.2

- There is a station at each of the 6 identified road crossings and 0 private property locations
- There is 1 TPDES outfall in this subwatershed; it is a potable water treatment facility permitted to discharge up to 200,000 gpd of filter backwash to a tributary of Pinkston Reservoir, thence to Sandy Creek, thence to West Creek
- Three private landowners have been contacted seeking their permission to allow site assessments on their property. To date, none of these have agreed to allow site access.

Naconiche Creek

Approximate Length = 29.8 miles
Number of Stations = 10
Average miles between Stations = 2.1

- There is a station at each of the 9 identified road crossings and 1 private property locations
- Five private landowners have been contacted seeking their permission to allow site assessments on their property. To date, none of these have agreed to allow site access.


Attoyac Bayou Watershed RUAA
Field Survey Sites
84
Rusk
FM 95
FM 1087
Naconiche Creek
59
Nacogdoches
Waffelow Creek
Terrapin Creek
Attoyac Bayou
FM 138
Shelby
West Creek
SH 7
Big Iron Ore Creek
96
San Augustine
FM 95
SH 21
N
Legend
Attoyac RUAA Sites
Big Iron Ore RUAA Sites
Naconiche RUAA Sites
Terrapin RUAA Sites
Waffelow RUAA Sites
West RUAA Sites
Assessment Units
Attoyac Bayou FRS
Lake Timpson
Permitted Dischargers
Pinkston Reservoir
Lake Naconiche
RUAA Water Bodies
Sam Rayburn Flowage Easement USACE
Watershed Streams
Texas Water Resources Institute
make every drop count
Location Map Generated by: Lucas Gregory 5.20.2011, modified 11.1.2011