

**Assessment of Water Quality and Watershed Planning
for the Leona River**

TSSWCB Project 11-50

Quality Assurance Project Plan

Revision No. 2

Prepared by

Texas Institute for Applied Environmental Research

Stephenville, Texas

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(with annual updates as applicable)

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A3 Distribution List

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LIST OF ACRONYMS

AWRL	Ambient Water Reporting Limit
BST	Bacterial Source Tracking
CAFO	Concentrated Animal Feeding Operation
CAR	Corrective Action Report
CMS	Coordinated Monitoring Schedule
COC	Chain-of-Custody
CRP	Clean Rivers Program
CWA	Clean Water Act
DOC	Demonstration of Capability
DQO	Data Quality Objective
DMRG	Data Management Reference Guide
EPA	U.S. Environmental Protection Agency
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus PCR
ERIC-RP	ERIC-PCR / RiboPrinting Combination Method
FY	Fiscal Year
GIS	Geographic Information System
GM	General Maintenance
LCS	Laboratory Control Sample
LCSD	Laboratory Control Sample Duplicate
LIMS	Laboratory Information Management System
LOD	Limit of Detection
LOQ	Limit of Quantitation
LQAO	Laboratory Quality Assurance Officer
NAIP	National Agriculture Imagery Program
NELAP	National Environmental Laboratory Accreditation Program
NIST	National Institute of Standards and Technology
NPS	Nonpoint Source
NRA	Nueces River Authority
NRCS	USDA Natural Resources Conservation Service
NWS	National Weather Service
PCR	polymerase chain reaction
PM	Project Manager
PQL	Practical Quantitation Limit
QA	Quality Assurance
QAM	Quality Assurance Manual
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
QC	Quality Control
QPR	Quarterly Progress Report
RL	Reporting Limit
RUAA	Recreational Use Attainability Analysis
SAML	Soil and Aquatic Microbiology Lab
SCSC	Department of Soil and Crop Sciences, Texas AgriLife Research
SOP	Standard Operating Procedure
SLOC	Station Location Request
SWQM	Surface Water Quality Monitoring
SWQMIS	Surface Water Quality Monitoring Information System
TIAER	Texas Institute for Applied Environmental Research
TMDL	Total Maximum Daily Load
TCEQ	Texas Commission on Environmental Quality

TSSWCB	Texas State Soil and Water Conservation Board
TSWQS	Texas Surface Water Quality Standards
UAA	Use Attainability Analysis
USGS	United States Geological Survey
WPP	Watershed Protection Plan
WWTF	Wastewater Treatment Facility

A4 Project/Task Organization

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

Ashley Alexander, TSSWCB Project Manager

Maintains a thorough knowledge of work activities, commitments, deliverables, and time frames associated with project. Develops lines of communication and working relationships between TIAER and TSSWCB. Tracks deliverables to ensure that tasks are completed as specified in the contract.

Responsible for ensuring that the project deliverables are submitted on time and are of acceptable quality and quantity to achieve project objectives. Participates in the development, approval, implementation, and maintenance of the QAPP. Assists the TSSWCB QAO in technical review of the QAPP. Responsible for verifying that the QAPP is followed by project participants. Notifies the TSSWCB QAO of particular circumstances that may adversely affect the quality of data derived from the collection and analysis of samples. Enforces corrective action.

Pamela Casebolt, TSSWCB Quality Assurance Officer

Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants. Responsible for verifying that the QAPP is followed by project participants. Determines that the project meets the requirements for planning, QA, QC, and reporting under the CWA §319(h) NPS Grant Program. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures.

Texas Institute for Applied Environmental Research, Tarleton State University, Stephenville, Texas – Responsible for general project oversight, coordination, administration, data collection, analyses and reporting, and development of project DQOs and QAPP.

Nikki Jackson, Project Manager

Responsible for implementing and monitoring TSSWCB requirements in contracts, QAPPs, and QAPP amendments and appendices. Coordinates project planning activities and work of project partners. Responsible for coordinating attendance at conference calls, training, meetings, and related project activities with the TSSWCB. Notifies the TSSWCB project manager of particular circumstances that may adversely affect the quality of data derived from the collection and analysis of samples. Enforces corrective action. Responsible for assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB Project Manager.

Jeff Stroebel, Field Operations Supervisor

Responsible for supervising all aspects of sample collection and handling, collection of field data, completion of field documentation, transportation of samples, and other field activities. Responsible for the acquisition of water samples, known source bacteria samples, and field data measurements in a timely manner that meet the DQOs specified in Section A7 (Table A7.1), as well as the requirements of Sections B1 through B8. Responsible for field scheduling, staffing, and ensuring that staff is appropriately trained as specified in Sections A6 and A8.

Nancy Easterling, Quality Assurance Officer

Responsible for coordinating development and implementation of TIAER's non-laboratory QA program. Participates in planning, development, approval, implementation, and maintenance of the QAPP. Responsible for maintaining records of QAPP distribution, including appendices and amendments. Responsible for identifying, receiving, and maintaining project QA records. Responsible for coordinating with the TSSWCB QAO to resolve QA-related issues. Notifies the TIAER Project Manager of particular circumstances that may adversely affect the quality of data. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Responsible for validation and verification of all TIAER generated data collected according to Table A7.1 and QC specifications. Coordinates the research and review of technical non-laboratory QA material and data related to water quality monitoring system design.

Mark Murphy, Laboratory Manager

Responsible for supervision of laboratory personnel involved in generating analytical data for this project, excluding BST data. For BST samples, responsible for coordinating preprocessing and shipping of samples to SCSC for analysis. Responsible for ensuring that laboratory personnel involved in generating analytical data have adequate training and a thorough knowledge of the QAPP and all SOPs specific to the analyses or task performed and/or supervised. Responsible for oversight of all operations, ensuring that all QA/QC requirements are met, and documentation related to the analysis is completely and accurately reported. Enforces corrective action, as required.

Mark Murphy, Laboratory QAO

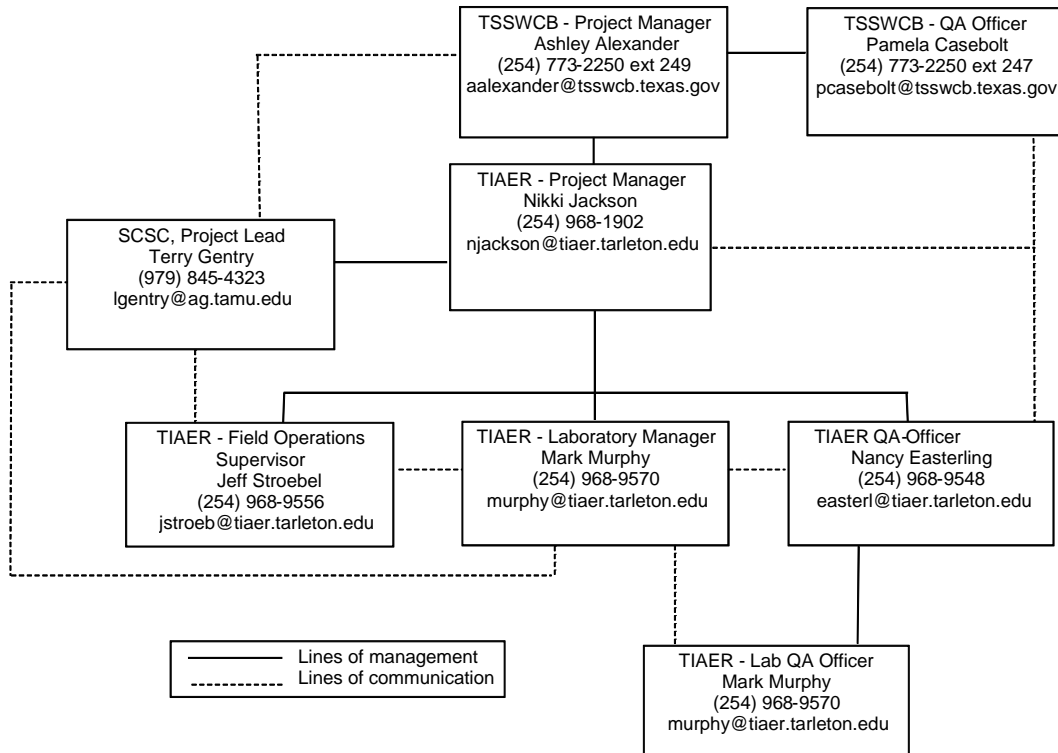
Monitors the implementation of the QAM and the QAPP within the laboratory to ensure complete compliance with QA objectives as defined by the contract and in the QAPP. Conducts internal audits to identify potential problems and ensure compliance with written SOPs. Responsible for supervising and verifying all aspects of the QA/QC in the laboratory. Performs validation and verification of data before data are evaluated to assess project objectives. Insures that all QA reviews are conducted in a timely manner from real-time review at the bench during analysis to final pass-off of data to the QAO. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems. Develops and facilitates internal monitoring systems audits.

Texas AgriLife Research, Department of Soil and Crop Sciences, Soil and Aquatic Microbiology Lab, College Station, Texas - Texas. Responsible for BST analysis and inclusion of fecal samples into the Texas *E. coli* BST Library.

Terry Gentry, Project Lead

Responsible for coordinating BST sample collection and preparation with TIAER and 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 measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to identify potential problems.

Figure A4.1. Project Organization Chart



Note: The NRA is also an important project partner, but the NRA is not involved with any direct data collection for the project, and, thus, not shown on the project organization chart for the monitoring QAPP.

A5 Problem Definition/Background

The Leona River (Segment 2109) is a tributary of the Frio River within the Nueces River Basin. The river flows 85 miles from US 83 in Uvalde County, through Zavala County, then to its confluence with the Frio River in Frio County (Figure A5.1). The watershed is approximately 429,244 acres. Cities within the watershed include Uvalde in Uvalde County and Batesville in Zavala County, both of which have wastewater discharge permits to the river.

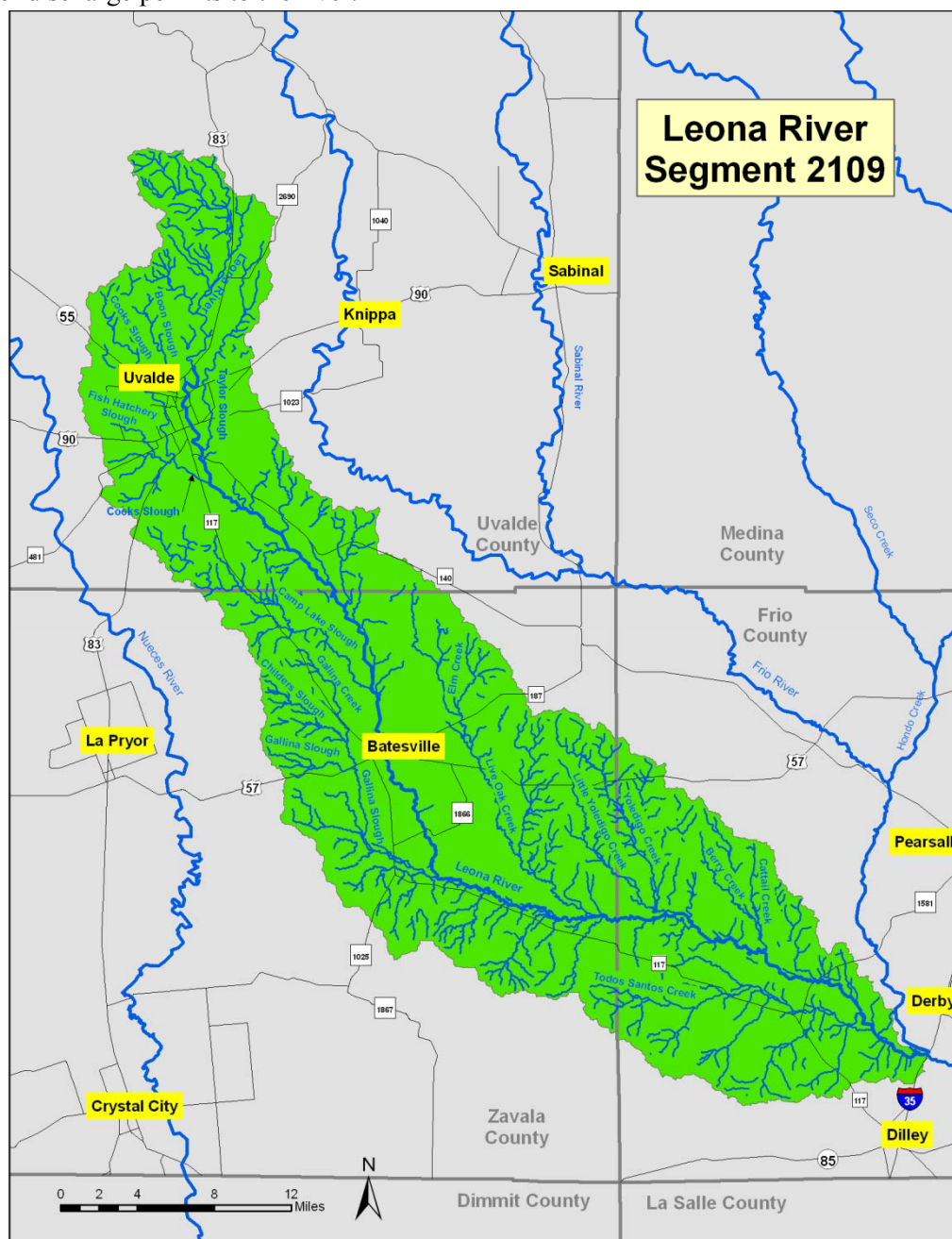


Figure A5.1. Map of Leona Watershed

The Leona River watershed is rural and land use is predominantly agriculture, including cropland and pastureland. According to the USDA National Agricultural Statistics Service 2007 Census of Agriculture, approximately 2.4 million acres of land in Frio, Uvalde, and Zavala counties are farmland. Leading animal operations that exist in all three counties are beef cattle and sheep. Winter wheat production, oats, sorghum and cotton are among the leading crops harvested in all three counties. Large amounts of land are also used to grow forages such as hay, grass silage and greenchop in Uvalde and Frio counties, and Frio County had more than 58,000 acres in peanut production in 2007.

While mainly rural, the cities of Uvalde and Batesville are located within the watershed. Uvalde has an estimated population of 16,000, while about 1,300 people reside in Batesville. Both cities have WWTFs with discharges into the Leona River; Uvalde actually has 2 outfalls. Other permitted dischargers include Agrilink Foods, which discharges processing waste via irrigation and the U.S. Fish and Wildlife Service National Fish Hatchery in Uvalde, which discharges flush water intermittently into the Leona.

The Leona River was first listed as having a bacteria impairment for contact recreation in the *2006 Texas Water Quality Inventory and 303(d) List*. It was listed as having a concern for bacteria in prior reports. It has also been listed as having a concern for nitrates beginning with the *2002 Texas Water Quality Inventory and 303(d) List*. The draft *2010 Texas Integrated Report* includes a bacteria impairment for all three AUs within the Leona River. The draft *2010 Texas Integrated Report* continues to note nitrates as a concern within all three AUs.

Historically, the Leona River was a popular place for swimming, canoeing, and fishing. Based on an editorial to the *Uvalde Leader News* on July 13, 2003, degradation began in the late 1960s. Increase runoff from agricultural fields, WWTF discharges, clearing of the riparian areas, and introduction of invasive plant species have all contributed to this degradation.

In 2004, NRA received a CWA §319(h) NPS Grant through the TSSWCB and the EPA, to design and implement an education program targeted at the headwater stream segments of the Nueces River Basin, including the Leona River. The Headwaters Stewardship program paved the way for an expanded sustained education effort by providing the education tools, enlightened audiences, and a cooperative capacity among local conservation organizations. This project will build on the success of the Headwaters Stewardship program.

The TCEQ and the TSSWCB established a joint, technical Task Force on Bacteria TMDLs in September 2006 charged with making recommendations on cost-effective and time-efficient bacteria TMDL development methodologies. The Task Force recommended the use of a three-tier approach that is designed to be scientifically credible and accountable to watershed stakeholders. The tiers move through increasingly aggressive levels of data collection and analysis in order to achieve stakeholder consensus on needed load reductions and strategies to achieve those reductions. In June 2007, the TCEQ and the TSSWCB adopted the principles and general process recommended by the Task Force and directed agency staff to incorporate the principles of the recommendations into projects that address bacteria impairments.

In accordance with the *Memorandum of Agreement between the TCEQ and the TSSWCB Regarding TMDLs, Implementation Plans, and WPPs*, the TSSWCB has agreed to take the lead role in addressing the bacteria impairments in this project's study area. Through this project, the TSSWCB and collaborating entities will work with local stakeholders to progress through the data collection and analysis components of the first two tiers of the Task Force recommended three-tier approach. The goal is to remove the waterbodies in the study area from the *303(d) List*; however, the mechanism is not predetermined. At the end of this two-year assessment project, possible outcomes include: 1) waterbodies are achieving current water quality standards, 2) adequate data exists to support a UAA to change water quality standards, 3) adequate data exists to develop a WPP, or 4) adequate data exists to develop a TMDL and I-Plan for TCEQ adoption.

A6 Project/Task Description

The overall goal of this project is to provide stakeholders and agencies with sufficient information to address bacteria impairments on the Leona River through verification of use attainment, revision of water quality standards, or development of a WPP or TMDL. This will be done through the following tasks as outlined in the project workplan:

- 1) Project Administration - To effectively administer, coordinate, and monitor all work performed under this project including technical and financial supervision and preparation of status reports.
- 2) Quality Assurance - To develop and implement DQOs and QA/QC activities to ensure data of known and acceptable quality are generated through this project.
- 3) Bacterial Source Tracking - To conduct BST to assess and identify different sources contributing to bacteria loadings.
- 4) Survey and Inventory Possible Bacteria Sources - To develop a comprehensive GIS inventory for the study area and to assess the possible sources of bacteria loadings by conducting a watershed source survey. To classify current land use for the watershed through a combination of satellite based image classification schemes and where needed “heads-up digitizing” of NAIP aerial photos of the area.
- 5) Surface Water Quality Monitoring - To provide sufficient water quality data to characterize bacteria and nitrate loadings across the various flow regimes at a number of locations throughout the study area.
- 6) Assess Attainability of Recreational Use - To collect information that can be used to evaluate factors affecting attainment of recreational use in the Leona River.
- 7) Data Analysis and Watershed Modeling - To analyze and interpret data using load duration curves and spatially explicit modeling to determine bacteria load reductions needed to achieve water quality standards and estimate loadings from various sources.
- 8) Public Participation and Stakeholder Facilitation - To facilitate public participation and coordinate stakeholder involvement to ensure that decision-making is founded on local input and that watershed action is successful.

For this project, TIAER will develop two separate QAPPs, one for water quality monitoring activities as addressed in Tasks 3, 5 and 6 of the workplan and one for watershed modeling activities addressed in Tasks 4 and 7 of the workplan. Because site selection for the RUAA under Task 6 necessitates extensive public involvement and coordination with other tasks, the RUAA and related items in Task 6 will be addressed in an amendment to the monitoring QAPP. Revision 0 of the QAPP addresses only the monitoring activities of this project under Tasks 3 and 5.

Task 3: Bacterial Source Tracking

Task 3 activities are related to BST. TIAER will collaborate with the SCSC, through TSSWCB project 10-50 *Support Analytical Infrastructure and Further Development of a Statewide Bacterial Source Tracking Library*, to conduct BST in the study area to assess and identify different sources contributing to bacteria loadings. Library-independent BST utilizing the *Bacteroidales* PCR genetic test will be combined with limited library-dependent BST utilizing the ERIC-RP combination method. The Texas *E.*

coli BST Library will also be supplemented with known fecal samples from the study area. In addition, the SCSC will assist TIAER in designing a watershed source survey, which will be conducted as part of Task 4 of the project. Direct data collection for the BST and known source samples is outlined below:

- The SCSC will conduct library-independent BST on 225 water samples collected by TIAER utilizing the *Bacteroidales* PCR genetic test for human, ruminant, horse, and swine markers. The number of samples may be adjusted depending on the complexity of sources as identified in the source survey (Task 4). 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, as the budget allows. Water samples for this subtask shall be a subset of those collected by TIAER through Task 5.
- The SCSC will conduct limited library-dependent BST and analyze *E. coli* isolates (1 isolate per water sample) from 75 water samples collected by TIAER from across the study area utilizing the ERIC-RP combination method (total of 75 *E. coli* isolates). Likely human and animal sources of the *E. coli* will be identified using the Texas *E. coli* BST Library. 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 *E. coli* BST Library. Water samples for this subtask shall be a subset of those collected by TIAER through Task 5.
- The Texas *E. coli* BST Library will be supplemented with known fecal samples from the study area. The SCSC will add up to 200 known source fecal samples (1-2 isolates per fecal sample) to the Texas *E. coli* BST Library from samples collected by TIAER. Fecal samples will be added to the Texas *E. coli* BST library utilizing the ERIC-RP combination method; isolates will be screened using ERIC-PCR and the non-clonal isolates (estimated at 20%) will be further analyzed using RiboPrinting. Samples for this subtask shall be collected by TIAER through Task 5.

The collection of BST and known source samples will be spread throughout the project, although an effort will be made to collect more samples early on in the project (particularly the known source samples) to avoid the risk of getting towards the end of the project and not having sufficient samples due to unexpected obstacles. The SCSC, in conjunction with the source survey, will work with TIAER and the TSSWCB to develop a target list of desired known sources. Results from the BST will be integrated into watershed modeling activities conducted by TIAER under Task 7. The SCSC will work with TIAER to integrate BST results into the model, to the extent possible, and address and reconcile discrepancies between BST and modeling results. The modeling effort for Task 7 will be addressed in a separate modeling QAPP for the project. The SCSC will be responsible for a final technical report of the results from the BST task and submittal of the BST data to the TSSWCB.

Task 5: Surface Water Quality Monitoring

Task 5 activities involve providing sufficient water quality data to characterize bacteria and nitrate loadings in the project area. To address this activity, TIAER will conduct routine ambient monitoring at mainstem, tributary and spring sites as well as monitor effluent at the outfalls of WWTFs in the watershed (Figure A6.1). Monitoring at all sampling stations will include *E. coli* enumerated using USEPA Method

1603, total nitrite+nitrate nitrogen, flow, and field parameters. Field parameters are pH, temperature, conductivity, and dissolved oxygen. Flow parameters are flow collected by gage, electric, mechanical or Doppler, including severity. TIAER will also establish, and maintain, three continuous flow monitoring gages (ISCO flowmeters), which will be located as close as practically possible to the outlet of each AU. Task 5 also includes a historical data review for the waterbody as well as coordination of collection and preprocessing of BST samples and know source fecal samples with Task 3. Direct data collections activities under Task 5 are outlined below:

- TIAER will conduct routine ambient monitoring at nine mainstem sites once every two weeks, collecting field, nitrite+nitrate nitrogen, flow, and bacteria parameter groups through the end of the project. The number of biweekly samples planned for collection is 404. Currently, routine ambient monitoring is conducted quarterly at three stations by TCEQ (12985, 12987, and 12989) and at one station by NRA (18418); TIAER will work with TCEQ and NRA to avoid duplicative routine ambient monitoring at these stations. Appropriate field splits at about 10% will be included for QA/QC for nitrogen analyses.
- TIAER will conduct targeted watershed monitoring at eight tributary sites once every month, collecting field, conventional, flow, and bacteria parameter groups through the end of the project. The number of samples planned for collection through this subtask is 154.
- TIAER will conduct routine effluent monitoring at three WWTF outfalls (two for the City of Uvalde and one for the City of Batesville) and one other permitted intermittent discharger (the Uvalde National Fish Hatchery) once every month, collecting field, conventional, flow and bacteria parameter groups through the end of the project. The number of samples planned for collection through this subtask is 88.
- TIAER will conduct monitoring at up to five springs or wells once per season collecting field, conventional, flow and bacteria parameter groups through the end of the project. The sampling period extends over eight seasons. The number of samples planned for collection through this subtask is 40.
- TIAER will establish, and maintain, continuous flow monitoring gages (ISCO flowmeters) at three mainstem sites through the end of the project. These sites shall be located near the outlets of each of the three AUs as is practically possible.
- TIAER will transfer monitoring data from mainstem and tributary sites to TSSWCB for inclusion in the TCEQ SWQMIS at least quarterly. Data will be transferred in the correct format using the TCEQ file structure, along with a completed Data Summary, as described in the most recent version of *TCEQ SWQM Data Management Reference Guide*. Other data (WWTFs, springs, continuous flow) will be transferred in an appropriate format.

As needed, TIAER will submit SLOCs to TCEQ to obtain TCEQ station numbers for new monitoring sites. TIAER will input the monitoring regime for mainstem, tributary, and spring sites into the TCEQ CMS. TIAER will be responsible for transferring data appropriate for SWQMIS to the TSSWCB on at

least a quarterly basis. Preliminary monitoring data will also be transferred in a timely manner to the NRA after TSSWCB review for posting on the project website as part of Task 8 dealing with public participation and stakeholder facilitation. TIAER will be responsible for two technical reports under this task, one dealing with historical and the other with current monitoring data that characterize trends and variability.

Task 6: Assess Attainability of Recreational Use

Task 6 focuses on RUAA for the Leona River. The RUAA will follow guidelines as outlined in the 2009 *TCEQ Procedures for a Comprehensive RUAA and a Basic RUAA Survey*. The RUAA includes a thorough historical review of information regarding recreational uses of the waterbody back to November 28, 1975 as well as direct surveys characterizing the length of the waterbody and public interviews. The direct data collection activities involved in Task 6 include the following:

- TIAER will conduct two field surveys at each selected site. These surveys shall be conducted during a normal warm season (air temperature $\geq 70^{\circ}\text{F}$) 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, and weekends).
- To ascertain the suitability of the stream for contact recreation use, field surveys shall document hydrological characteristics of the stream, such as width and depth of channel and substantial pools, flow/discharge, air/stream temperature, bank access, and stream substrate. Information to be collected shall at least satisfy those questions found on the Field Data Sheet in Appendix G.
- TIAER shall document and describe antecedent (prior to fieldwork) rainfall conditions (approximately 30 days) at each selected site.
- TIAER 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.
- In order to obtain information on existing and historical uses and stream characteristics, TIAER 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, where possible. Surveys shall include at least those questions found on the Interview Form in Appendix G.

Findings from historical information review, field surveys, and user interviews will be combined by TIAER into a technical report that shall at least include those contents described for a Comprehensive RUAA in the 2009 version of the *TCEQ Procedures for a Comprehensive RUAA and a Basic RUAA Survey*.

Revisions to the QAPP

Until the work described is completed, this QAPP shall be revised as necessary and reissued annually on

the anniversary date of QAPP approval, or revised and reissued within 120 days of significant changes, whichever is sooner. The most recently approved QAPP shall remain in effect until revisions have been fully approved; re-issuances (i.e., annual updates) must be submitted to the TSSWCB for approval before the anniversary date. If the entire QAPP is current, valid, and accurately reflects the project goals and organization's policy, the annual reissuance 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

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 for amendments are directed from the TIAER Project Manager to the TSSWCB Project Manager in writing. The changes are effective immediately upon approval by the TSSWCB Project Manager and Quality Assurance Officer.

Amendments to the QAPP and the reasons for the changes will be documented, and revised pages will be forwarded to all persons on the QAPP distribution list by the TIAER QAO. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process or within 120 days of the initial approval in cases of significant changes.

A7 Quality Objectives and Criteria

The objective of this section is to ensure that data collected meets the DQOs of the project. The major objective is to identify specific sources of bacteria entering the Leona River. A secondary objective is to evaluate sources of nitrates. At the end of this two-year assessment project, possible outcomes include: 1) waterbodies are achieving current water quality standards, 2) adequate data exists to support a UAA to change water quality standards, 3) adequate data exists to develop a WPP, or 4) adequate data exists to develop a TMDL and I-Plan for TCEQ adoption.

Surface Water Quality Monitoring

Bacterial pollution within the Leona River Watershed will be assessed through water quality monitoring of sampling stations in the Leona River, designated tributaries, permitted dischargers, and spring sites. Measurement performance criteria to support the project objective are specified in Table A7-1.

Routine ambient grab samples will be collected at the stations listed in Table B1.1. Samples will be collected once every two weeks at the nine mainstem stations, monthly at the eight tributary stations and four permitted discharge stations, and quarterly at the five spring stations. Stream stations will be sampled if flowing or if there are perennial pools. If a pool is sampled, the flow severity equals 1 for no flow and basic information regarding the size of the pool should be recorded, including maximum pool width (meters), maximum pool depth (meters), pool length, and the percent of pool coverage in a 500 m reach, where possible. During routine sampling, measurements of DO, conductivity, pH, water temperature, estimated flow severity, and stream flow will be obtained in situ. Flow for spring sites will be measured directly, if conditions allow. Flow at spring sites may be difficult if not impossible to distinguish from streamflow, when stream levels are above the level at which spring water enters the stream. Flow measurements of the permitted discharges will be measured directly, but they may be obtained from the discharge facility if circumstances in the field preclude direct measurement. Flow will not be measured from well sites, because measurement of groundwater flow is beyond the capabilities of this study. Field data will be logged on field data sheets, incorporated into a computer based database maintained by TIAER.

Water samples will be analyzed by the TIAER Laboratory for total nitrite-nitrate nitrogen and *E. coli* within designated holding times using methods specified 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 TIAER laboratory will receive water and known source fecal samples for subsequent preprocessing for BST analyses and shipping to SCSC as outlined in Sections B1, B2 and B3.

BST Analysis

The objective of this portion of the project is to assess and identify different sources contributing to bacteria loadings in the Leona River 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.

Table A7.1 Measurement Performance Specifications for Routine Water Quality Monitoring

Parameter	Units	Matrix	Method	Parameter Code	AWRL	Limit of Quantitation	LOQ Ck Std % Recovery	Precision	Bias (LCS % Rec.)	Lab
Field Parameters										
pH	pH/ units	water	EPA 150.1 and TCEQ SOP, V1	00400	NA	NA	NA	NA	NA	Field
DO, dissolved oxygen	mg/L	water	EPA 360.1 and TCEQ SOP, V1	00300	NA	NA	NA	NA	NA	Field
Specific Conductance	µS/cm	water	EPA 120.1 and TCEQ SOP, V1	00094	NA	NA	NA	NA	NA	Field
Temperature	°C	water	EPA 170.1 and TCEQ SOP V1	00010	NA	NA	NA	NA	NA	Field
Flow	cfs	water	TCEQ SOP V1	00061	NA	NA	NA	NA	NA	Field
Days since last precipitation	Days	water	TCEQ SOP V1	72053	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
Flow measurement method	1-gage 2-electric 3-mechanical 4-weir/flume 5-doppler	other	TCEQ SOP V1	89835	NA	NA	NA	NA	NA	Field
Maximum pool width	meters	water	TCEQ SOP V1	89864	NA	NA	NA	NA	NA	Field
Maximum pool depth	meters	water	TCEQ SOP V1	89865	NA	NA	NA	NA	NA	Field
Pool length	meters	water	TCEQ SOP V1	89869	NA	NA	NA	NA	NA	Field
% pool coverage in 500 meter reach	%	water	TCEQ SOP V1	89870	NA	NA	NA	NA	NA	Field
Conventional Laboratory Parameters										
<i>E. coli</i> mTEC	CFU/100 mL	water	USEPA 1603	31648	1	1	NA	0.5 ¹	NA	TIAER
NO ₂ -N+NO ₃ -N, Nitrite/nitrate-N total	mg/L	water	SM online 4500-NO ₃ -F	00630	0.05	0.05	70-130	20	80-120	TIAER

¹ Based on range statistics described in *Standard Methods for the Examination of Water and Wastewater*, Online Edition, Section 9020-B “QA/QC – Intralaboratory QC Guidelines.” This criterion applies to bacteria duplicates with concentrations >20 CFU/100mL.

References:

USEPA *Methods for Chemical Analysis of Water and Wastewater*, Manual # EPA-600/4-79-020.
American Public Health Association, American Water Works Association and Water Environment Federation, *Standard Methods for the Examination of Water and Wastewater*, online Ed.
TCEQ *Surface Water Quality Monitoring (SWQM) Procedures, Volume 1: Physical and Chemical Monitoring Methods*, latest version (RG-415) and updates issued by TCEQ

Table A7.2 Measurement Performance Specifications for BST

BST Parameters							
Parameter	Method Type	Method	Method Description	Precision of Lab Duplicates¹	Bias	% Complete²	Lab
<i>E. coli</i> RiboPrinting	DNA/image matching	SCSC SOP	RiboPrinting for library dependent <i>E. coli</i> isolates from water samples and known source fecal samples	90% identical	90% correct	90%	SCSC
<i>E. coli</i> ERIC-PCR fingerprint	DNA/image matching	SCSC SOP	ERIC-PCR for library dependent <i>E. coli</i> isolates from water samples and known source fecal samples	90% identical	90% correct	90%	SCSC
<i>Bacteroidales</i> PCR	PCR presence/absence	SCSC SOP	<i>Bacteroidales</i> PCR for library independent water samples	100% agreement	90% correct	90%	SCSC
<i>E. coli</i> isolation	membrane filter culture on modified mTEC agar	USEPA 1603	Membrane filter for library dependent <i>E. coli</i> isolates from water samples	NA	NA	NA	TIAER
<i>E. coli</i> isolation	membrane filter culture on modified mTEC agar	USEPA 1603	Membrane filter for known source fecal samples	NA	NA	NA	SCSC

¹ Bias and laboratory method precision will be determined using isolates from known source samples in a blind procedure, as discussed in Section B5.

² The objective is for 90% of the data to be collected. An additional objective for BST completeness is that sources for 70% of host-specific isolates can be identified.

RUAA

The objective of this portion of the project is to collect data that may be used to support decisions related to the recreational use designation of the Leona River. The specific data to be collected from each site during each survey are listed on the Field Data Sheet included in Appendix G. Measurements to be collected are listed in Table A7.3 for the RUAA. Of note, these measurements are to be used for the RUAA survey only and are not intended to be submitted as data for SWQMIS.

Table A7.3 Measurement Performance Specifications for RUAA Field Data

Parameter	Units	Matrix	Method	Stream Type	Parameter Code	Lab
Air Temperature	°C	Air	EPA 170.1 & TCEQ SOP	NA	00020	Field
Water Temperature	°C	Water	EPA 170.1 & TCEQ SOP	NA	00010	Field
Flow	cfs	Water	TCEQ SOP	NA	00061	Field
Flow Measurement Method	1-gage, 2-electric, 3-mechanical, 4-weir/flume, 5-doppler	Water	TCEQ SOP	NA	89835	Field
Length, Width & Depth for Substantial Pool	meters	Water	Basic RUAA Survey	Wadeable	NA	Field
Thalweg Depth	meters	Water	Basic RUAA Survey	Wadeable	NA	Field
Stream Width	meters	Water	Basic RUAA Survey	Wadeable and non-wadeable	NA	Field

References:

USEPA *Methods for Chemical Analysis of Water and Wastewater*, Manual # EPA-600/4-79-020.
American Public Health Association, American Water Works Association and Water Environment Federation, *Standard Methods for the*

Examination of Water and Wastewater, online Ed.
TCEQ Surface Water Quality Monitoring (SWQM) Procedures, Volume 1: Physical and Chemical Monitoring Methods, latest version (RG-415)
and updates issued by TCEQ.

Limit of Quantitation

Ambient Water Reporting Limits (AWRLs) are used for conventional laboratory parameters in this project as the limit of quantitation specification, so data collected under this QAPP can be compared against the TSWQS. Laboratory limits of quantitation (Table A7.1) must be at or below the AWRL for each applicable parameter.

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 QC 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. Field splits will be collected only for total nitrite+nitrate nitrogen samples and not for bacteria samples due to need to avoid sample contamination.

For bacteria analysis, laboratory precision is assessed by comparing replicate analyses of sample/duplicate pairs. Precision for bacteria is calculated by determining the range (R_{bacteria}), which is defined as the difference between the base-10 logarithm of sample duplicates. 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. For quantitative microbiological analyses, the method to be used for calculating precision is

the one outlined in *Standard Methods for the Examination of Water and Wastewater*, online edition, section 9020 B.8.b.

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

The ERIC-PCR and RiboPrinting BST techniques are qualitative assays, generating two different types of DNA fingerprints. Precision for ERIC-PCR and RiboPrinting will be determined using a control strain of *E. coli* (QC101) and *E. coli* from known-source samples, with a goal of 85% precision for the combined ERIC-RP fingerprint.

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., deionized 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

Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions typical of a waterbody. Site selection, the appropriate sampling regime, the sampling of all pertinent media, and use of only approved analytical methods will assure that the measurement data represents the conditions at the site. Water quality data that are collected on a routine frequency are separated by approximately even time intervals. Although data may be collected during varying regimes of weather and flow, the data sets will not be biased toward unusual conditions of flow, runoff, or season. The goal for meeting total representation of the waterbody will be tempered by the potential funding for complete representativeness.

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 that 90% data completion is achieved.

Comparability

Confidence in the comparability of data sets for this project is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP. Comparability is also guaranteed by reporting data in standard units, by using accepted rules for rounding figures, and by reporting data in a standard format as specified in Section B10.

A8 Special Training/Certification

Surface Water Quality Monitoring

Field personnel receive training in proper sampling and field analysis. Before actual sampling or field analysis occurs, they will demonstrate to the QAO (or designee) their ability to properly calibrate and operate field equipment. Field personnel training is documented and retained in the personnel file and will be available during a monitoring systems audit.

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. SCSC personnel involved in this project have been trained in the appropriate use of laboratory equipment, laboratory safety, cryogenics safety, and all applicable SOPs. The SCSC will also provide, as necessary, guidance and training to TIAER personnel regarding the preprocessing of BST samples. The SCSC is NELAP certified for *E. coli* analysis, used for this project by the laboratory in growing cultures for known source fecal samples. TIAER is NELAP certified for *E. coli* analysis, used in isolating *E. coli* as part of the pre-processing procedure prior to BST analysis.

Water Quality Analysis

Analyses performed by the TIAER Laboratory for total nitrite+nitrate nitrogen and *E. coli* (USEPA Method 1603) for this project have NELAP accreditation. All analysts have a current DOC for the analyses they perform for the project.

RUAA

Field personnel will have received training in procedures for collecting measurement and survey data associated with an RUAA as outlined in the 2009 *TCEQ Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey* prior to conducting RUAA surveys.

A9 Documents and Records

Surface Water Quality Monitoring

Hard copies of all field data sheets, general maintenance (GM) records, COC forms, laboratory data entry sheets, field data entry sheets, calibration logs, and CARs will be archived by TIAER for at least five years after close of the project. In addition, TIAER will archive electronic forms of all project data for at least five years. Examples are presented of TIAER GM (used with ISCO flowmeters) and field data sheets in Appendix A and the TIAER COC form in Appendix B.

BST Analysis

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

RUAA

Forms and information to be maintained for the RUAA include the following as found in Appendix G:

- Contact Information Form (electronic format)
- Field Data Sheets – Basic RUAA Survey (electronic and scanned field notes, if hand entered)
- Comprehensive RUAA Interview Forms (electronic and scanned, if hand entered)
- Digital photographic record, cataloged in an appropriate manner.

Project Documentation

TIAER will electronically produce QPRs for the TSSWCB combining information from all project partners and will note activities conducted in connection with audits of the water quality monitoring program, items or areas identified as potential problems (e.g., CARs impacting data quality), and any variations or supplements to the QAPP.

CARs will be utilized when necessary (Appendix C). CARs will be maintained in an accessible location for reference at TIAER. 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, when appropriate.

Individuals listed in Section A3 at TIAER and SCSC will be notified of approval of the most current copy of the QAPP by the TIAER PM. The TIAER PM will ensure the distribution of the most recent version of the QAPP to those on the A3 list.

The final project reports will be produced electronically and as a hard copy and all files used to produce the final report will be saved electronically by TIAER and SCSC for at least five years.

The documents and records that describe, specify, report, or certify activities are listed in Table A9.1. The TSSWCB may elect to take possession of records at the conclusion of the specified retention period.

Table A9.1 Records and Documents Retention Requirements

Document/Record	Location	Retention	Format
QAPPs, amendments and appendices	TIAER QAO Offices	5 years	Paper
QAPP, distribution documentation	TIAER QAO Offices	5 years	Paper
Field training records	TIAER Field Offices	5 years	Paper
Field notebooks or data sheets	TIAER Field Offices	5 years	Paper
Field equipment calibration/maintenance logs	TIAER Field Offices	5 years	Paper
Field instrument printouts	TIAER Field Offices	5 years	Paper
Field SOPs	TIAER Field Offices	5 years	Paper
Chain of custody records	TIAER Data Management Offices	5 years	Paper
Laboratory Quality Manuals	TIAER and SCSC Laboratories	5 years	Paper
Laboratory SOPs	TIAER and SCSC Laboratories	5 years	Paper
Laboratory training records	TIAER and SCSC Laboratories	5 years	Paper
Laboratory instrument printouts	TIAER and SCSC Laboratories or Offsite Storage	5 years	Paper
Lab equipment maintenance logs and calibration records	TIAER and SCSC Laboratories or Offsite Storage	5 years	Paper
Laboratory data reports/results	TIAER and calibration records or Offsite Storage	5 years	Paper/ electronic
Corrective Action Documentation	TIAER and SCSC offices	5 years	Paper/ electronic
RUAA Contact Information, Field Data and Interview Forms	TIAER Field Offices	5 years	Paper/ electronic
RUAA Photographs	TIAER Field Offices	5 years	Electronic

As an electronic data protection strategy, TIAER utilizes Double Take software to mirror the Primary Aberdeen 1.2TB file server TIAER5A located in Hydrology 2nd floor (* RAID 5 fault tolerant) that will be mirrored to a secondary Aberdeen Abernas211 file server TIAER5B located in Davis Hall 4th floor (* RAID 5 fault tolerant). This provides instant fault recovery rollover capability in the event of hardware

failure. TIAER also exercises complete backup of its Primary server to LTO-3 Quantum ValueLoader on a weekly basis, coupled with daily incremental backups. This provides a third level of fault tolerance in the event that both the primary and secondary servers are disabled. TIAER will maintain all cyclic backup tapes for 26 weeks prior to reuse saving the 1st tape in the series indefinitely to preserve a historical snapshot. This will facilitate recovery of data lost due to human error. Backup tapes are stored in a secure area on the Tarleton State University campus and are checked periodically to ensure viability. If necessary, disaster recovery can also be accomplished by manually re-entering the data.

At SCSC, all electronic data are backed up on an external hard drive monthly, compact disks weekly, and simultaneously saved in an external network folder and the computer's hard drive.

Laboratory Documentation

The TIAER laboratory will document sample results clearly and accurately. Information about each water quality sample will include the following to aid in interpretation and validation of data:

- A clear identification of samples analyzed for the project including station information
- Date and time of sample collection
- Identification of preservation and analysis methods used
- Sample results, units of measurement, and sample matrix
- Information on QC failures or deviations from requirements that may affect the quality of results or is necessary for verification and validation of data

Electronic Data

All monitoring data will be submitted to the TSSWCB at least quarterly for review prior to placement on the project website maintained by the NRA. Project data on the website will be considered preliminary and noted as subject to change.

All field, flow, total nitrite+nitrate-nitrogen, and mTEC *E. coli* data for the mainstem and tributary stations will also be submitted to the TSSWCB at least quarterly in the event/result format specified in the TCEQ DMRG for upload to SWQMIS. The Data Summary checklist required by the TCEQ will be submitted with the data. The routine stream data will be submitted under monitoring type RT. Data collection sites for this project have been or will be assigned a Station Identification Number by TCEQ.

Submitting Entity, Monitoring Entity, and Monitoring Type will reflect the entity reporting the data, the entity collecting the data, and the data collection targeted toward NPS data as follows:

Sample Description	Submitting Entity	Monitoring Entity	Monitoring Type
Routine <i>E. coli</i> , total nitrite+nitrate nitrogen, field and flow data from mainstem and tributary stations	TSSWCB (TX)	TIAER (TA)	RT

For BST data, a final EXCEL spreadsheet will be submitted by the SCSC to the TSSWCB.

B1 Sampling Process Design

To provide sufficient water quality data to characterize bacteria and nitrogen loadings across the various flow regimes, TIAER will conduct routine ambient monitoring at nine mainstem stations once every two weeks, monthly at the eight tributary and four permitted discharge stations, and quarterly at up to five spring stations (Figure B1.1 and Table B1.1). TIAER will establish and maintain continuous flow monitoring gages (ISCO flowmeters) at three mainstem sites located as near to the outlets of each of the three AUs as is practically possible. Continuous stage recordings at these three stations will extend over the duration of the project.

Coordination between TPDES permittees and the TCEQ Regional Office will be required. Neither TIAER nor NRA nor TSSWCB shall submit WWTF data to TCEQ for use in permit compliance and enforcement; rather, WWTF data will only be used to estimate bacteria loadings from wastewater discharges and to assist TPDES permittees in improving management and operations.

Field data and samples will be collected following procedures detailed in the *TCEQ SWQM Procedures, Volume 1: Physical and Chemical Monitoring Methods (RG-415)* including updates issued by TCEQ. In order to obtain representative results, ambient water sampling will occur on a routine schedule throughout the project, 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. In the instance that a sampling site (Table B1.1) is inaccessible, no sample will be taken and the reason the site was inaccessible will be documented on the field data sheet.

In addition, fecal material samples from known sources will be collected for use in validating the BST methodologies. Up to 200 known source samples will be collected throughout the course of the project as time and opportunity arise for the field crew, while in the study area. These samples will include domestic animals, wildlife and human sources. A target list of specific known sources will be developed by the SCSC as the source survey for the project is designed and developed. 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. Therefore, specific locations or coordinates cannot be listed prior to sample collection of this nature, but if possible, will be recorded as additional information for each sample. Sample handling and shipping procedures are provided in Appendix D.

Samples collected by TIAER field staff will be submitted to the TIAER Laboratory, which will provide the known source fecal samples and a subset of *E. coli* and water samples to SCSC for BST analysis (Table B1.1). SCSC will perform library-independent BST utilizing *Bacteroidales* PCR on approximately 225 individual water samples, preprocessed by TIAER via filtration with the filters shipped to SCSC. SCSC will also isolate and fingerprint (ERIC-RP) 75 *E. coli* isolates (from 75 individual samples) collected from a subset of the same project sampling stations and times from which library-independent samples were collected (i.e., all library-dependent samples should have a complementary library-independent sample). Samples for BST analysis will be collected throughout the study, but an effort will be made to collect more of these samples early on in the project to avoid the risk of an insufficient sample numbers towards the end of the project.

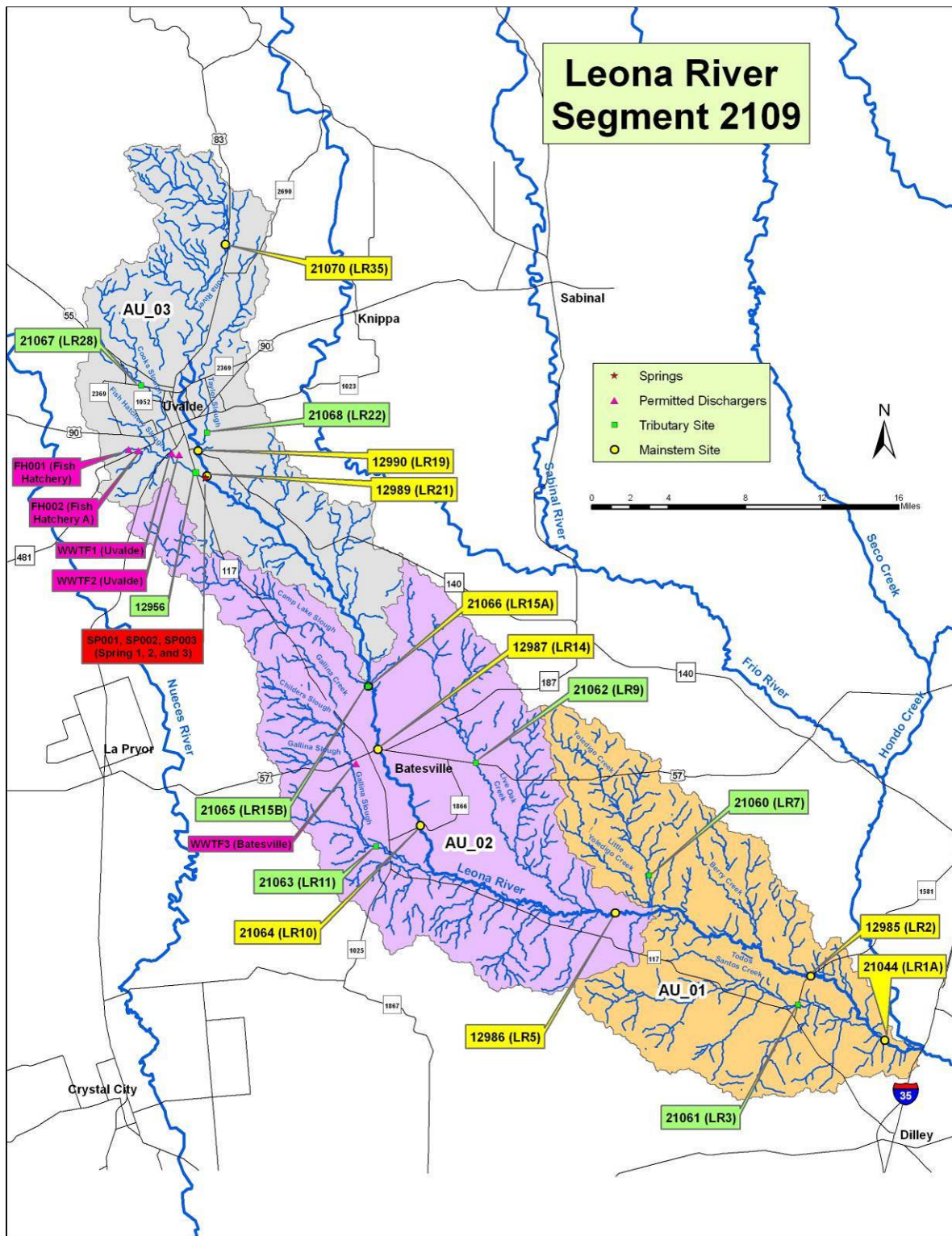


Figure B1.1. Map of project monitoring sites. Temporary station identification numbers used prior to issuance of TCEQ station identification numbers are listed in parentheses for mainstem and tributary sites.

Table B1.1 Monitoring Sites and Monitoring Frequencies

Segment AU	Site ID ¹	Site Description	Latitude	Longitude	Station Type	Monitoring Frequency (B=once every 2 weeks ² , M=monthly, Q=quarterly, C=continuous)				
						Field	Flow	<i>E. coli</i>	Nitrate	Contin uous Stage Height
2109_01	21061 (LR3)	Todos Santos Creek on FM 1581	28.7709	-99.252073	Tributary	M	M	M	M	
2109_01	12985 (LR2)	Leona River on FM 1581; CRP site	28.793011	-99.241125	Mainstem	B	B	B	B	
2109_01	21060 (LR7)	Yoledigo Creek on CR 4757	28.868586	-99.380567	Tributary	M	M	M	M	
2109_01	21044 (LR1A)	Leona River on Private Property near confluence with Frio River	28.744411	-99.177144	Mainstem	B	B	B	B	C
2109_02	12986 (LR5)	Leona River at Loma Vista Road (CR 4757)	28.8405	-99.407627	Mainstem	B	B	B	B	C
2109_02	21062 (LR9)	Live Oak Creek on US 57	28.953346	-99.529863	Tributary	M	M	M	M	
2109_02	21064 (LR10)	Leona River on FM 1866, USGS gauge #08204250	28.905851	-99.577419	Mainstem	B	B	B	B	
2109_02	21063 (LR11)	Gallinna Slough on CR 117	28.889567	-99.615624	Tributary	M	M	M	M	
2109_02	12987 (LR14)	Leona River at US 57 near Batesville: CRP site	28.963631	-99.614258	Mainstem	B	B	B	B	
2109_02	21065 (LR15B)	Camp Lake Slough near CR 1005B near confluence with Leona River	28.889567	-99.615624	Tributary	M	M	M	M	

Segment AU	Site ID ¹	Site Description	Latitude	Longitude	Station Type	Monitoring Frequency (B=once every 2 weeks ² , M=monthly, Q=quarterly, C=continuous)				
						Field	Flow	<i>E. coli</i>	Nitrate	Contin uous Stage Height
2109_03	21066 (LR15A)	Leona River on private property near CR 1005B; just above confluence of Camp Lake Slough	29.011228	-99.622832	Mainstem	B	B	B	B	C
2109_03	12990 ⁵ (LR19)	Leona River at FM 140 (near 117 intersection)	29.188787	-99.77098	Mainstem	B	B	B	B	
2109_03	12989 (LR21)	Leona River at Hoags Dam, below confluence of Cooks Slough; CRP site	29.170088	-99.763183	Mainstem	B	B	B	B	
2109_03	21068 (LR22)	Taylor slough on CR 373	29.202391	-99.763276	Tributary	M	M	M	M	
2109_03	12956	Cooks Slough at FM 117	29.171288	-99.772269	Tributary	M	M	M	M	
2109_03	21067 (LR28)	Cook Slough at FM 2369	29.237963	-99.820551	Tributary	M	M	M	M	
2109_03	21070 (LR35)	Leona River at CR 429A near Uvalde, TX; USGS gauge #08203450	29.345301	-99.74868	Mainstem	B	B	B	B	
2109_03	WWTF1	Uvalde WWTF discharge 1	29.18637	-99.792876	WWTF	M	M	M	M	
2109_03	WWTF2	Uvalde WWTF discharge 2	29.185775	-99.786987	WWTF	M	M	M	M	
2109_02	WWTF3	Batesville WWTF discharge	28.952399	-99.632568	WWTF	M	M	M	M	

Segment AU	Site ID ¹	Site Description	Latitude	Longitude	Station Type	Monitoring Frequency (B=once every 2 weeks ² , M=monthly, Q=quarterly, C=continuous)				
						Field	Flow	E. coli	Nitrate	Contin uous Stage Height
2109_03	FH001 ³ (Fish Hatchery)	Uvalde National Fish Hatchery discharge	29.189378	-99.830521	Discharge	M	M	M	M	
2109_03	FH002 ³ (Fish Hatchery A)	Alternative site for collection of Fish Hatchery discharge	29.188703	-99.821891	Discharge ³	M	M	M	M	
2109_03	SP001 (Spring 1)	Spring 1 near 12989	29.168631	-99.764123	Spring	Q	Q	Q	Q	
2109_03	SP002 (Spring 2)	Spring 2 near 12989	29.168046	-99.764087	Spring	Q	Q	Q	Q	
2109_03	SP003 ⁴ (Spring 3)	Spring 3 near 12989	29.167669	-99.763804	Spring	Q	Q	Q	Q	

¹ Temporary station identification numbers used prior to issuance of TCEQ station identification numbers are listed in parentheses for mainstem and tributary sites.

² Once every 2 weeks monitoring at mainstem sites will be coordinated with routine quarterly monitoring conducted under the CRP by the NRA or TCEQ, which occurs at monitoring stations 12985, 12987, 12989 and 18418⁵.

³ Federal approval will be needed for monitoring of discharge directly from the Uvalde National Fish Hatchery. An alternate location just prior to where the discharge enters Fish Hatchery Slough is recommended while awaiting federal approval.

⁴ Only three spring sites currently identified.

⁵ Sites 18418 and 12990 are within 300 meters of each other

For the RUAA survey, the sampling process design is guided by information in the 2009 *TCEQ Procedures for a Comprehensive RUAA and a Basic RUAA Survey*. TIAER will conduct field surveys at selected sites during periods when people would be most likely to use the waterbody for contact recreation. The RUAA surveys will ascertain the suitability of the Leona River for contact recreation use and document the hydrological characteristics of the river. Field data will be collected following procedures detailed in *TCEQ SWQM Procedures Volume 1: Physical and Chemical Monitoring Methods*, 2012 (RG-415).

The Leona River (Segment 2109) is just over 90 river miles long, which indicates a goal of 54 sites (3 sites per 5 miles of river) for the RUAA survey. 34 sites were selected for the RUAA, 15 of which are publically accessible via road crossing or parks and 19 of which are accessible via private property (Table B1.2). Public access to the Leona River is generally limited to a few road crossing, parks within the Cities of Uvalde and Batesville, and through the Fort Inge Historical Park located south of Uvalde, which is operated by the Uvalde Historical Commission and open only on weekends and for special events. Sites with public access associated with parks include AU03_09, which will allow the reach through the Uvalde Municipal Park and Golf Course to be assessed; AU03_06 and AU03_07 within Ft Inge; and AU02_10 within the Batesville City Park. Other sites with public access were associated with road

crossings. Landowner permission has been granted to access the sites that are on private property. Table B1.2 and Figure B1.2 below indicate the RUAA survey stations for the Leona River and are labeled based on their location by assessment unit (AU01, AU02 and AU03) in a downstream to upstream order.

With regard to assessing the impact of permitted discharges on the Leona River, there are two permitted WWTFs within the Leona River watershed. The City of Uvalde WWTF has three permitted outfalls. The primary outfall is located at the facility and discharges into a series of ponds developed as a wetlands area and then into Cooks Slough, a tributary of the Leona River. Effluent from the Uvalde WWTF is often diverted to a second outfall that discharges directly into the Leona River at a point within the Uvalde City Park. A third outfall for the City of Uvalde WWTF, which is seldom used based on discharge records, is located near the facility and directly discharges into Cooks Slough bypassing the wetland ponds. RUAA sites have been selected that reflect the water of the Leona River above and below the confluence of Cooks Slough as well as a site within the Uvalde City Park to capture the impacts of the Uvalde WWTF outfalls (Table B1.2). The City of Batesville is unincorporated and the WWTF is operated by the Batesville Water Supply Corporation. The effluent from the Batesville WWTF generally evaporates in holding ponds, but if discharge were to occur, it would flow into Gallina Slough, a major tributary of the Leona River located in AU02 (Figure B1.2). Other dischargers with active permits include the U.S. Fish and Wildlife Service National Fish Hatchery in Uvalde, which discharges flush water intermittently into Fish Hatchery Slough, which joins the Leona River via Cooks Slough west of Uvalde in AU03; the Chaparral Cattle Feedlot CAFO located south of Uvalde in AU03; and the Live Oak Feedlot located southeast of Batesville within the watershed of Liveoak Creek CAFO in AU02. A vegetable processing plant operated by TAFMI, Inc. (previously Agrilink Foods) located north of Uvalde used to be permitted to discharge wastewater via land irrigation, but according to TCEQ records, this operation no longer has an active permit, thus, it is not included on the maps provided.

Table B1.2. Leona River Segment 2109 RUAA Sites

Sites are listed in downstream to upstream order along the segment.

TCEQ ID (if collocated)	Site ID	Site Description	Latitude (NAD83)	Longitude (NAD83)	Estimated Distance to Previous Station (miles) ¹	Estimated Distance from Upper Segment Boundary (miles) ¹	Estimated Distance from Lower Segment Boundary (miles) ¹	Private or Public Access	Private Access Landowner Approved
---	---	[SEGMENT & AU01 lower boundary at confluence with Frio River]	---	---	---	91.04	0.00	---	---
---	AU01_01	Leona River near the confluence of the Leona River with the Frio River	28.738116	-99.146804	0.11	90.93	0.11	Private	Yes
21044	AU01_02	Leona River about 3.5 river miles above the confluence with the Frio River	28.744411	-99.177144	3.40	87.53	3.51	Private	Yes
---	AU01_03	Leona River about 4.7 river miles above the confluence with the Frio River	28.751352	-99.188358	1.18	86.35	4.69	Private	Yes
---	AU01_04	Leona River about 5.6 river miles above the confluence with the Frio River	28.757464	-99.192778	0.94	85.41	5.63	Private	Yes
---	AU01_05	Leona River below confluence of Todos Santo Creek	28.765985	-99.200515	1.66	83.75	7.29	Private	Yes
---	AU01_06	Leona River about 9.2 river miles above the confluence with the Frio River	28.781534	-99.211765	1.92	81.83	9.21	Private	Yes

¹ Distances were digitally estimated using the measuring tool in ArcGIS 9.3 with the 2010 NAIP 1m DOQQs and the NHD stream layer as reference guides.

TCEQ ID (if collocated)	Site ID	Site Description	Latitude (NAD83)	Longitude (NAD83)	Estimated Distance to Previous Station (miles) ¹	Estimated Distance from Upper Segment Boundary (miles) ¹	Estimated Distance from Lower Segment Boundary (miles) ¹	Private or Public Access	Private Access Landowner Approved
---	AU01_07	Leona River about 11 river miles above the confluence with the Frio River	28.790568	-99.223338	1.87	79.96	11.08	Private	Yes
12985	AU01_08	Leona River at FM 1581	28.793011	-99.241125	2.20	77.76	13.28	Public	---
---	AU01_09	Leona River west of FM 1581	28.794222	-99.251737	0.83	76.93	14.11	Private	Yes
---	---	[AU01 upper / AU02 lower boundary at confluence of Yoledigo Creek]	---	---	15.46	61.47	29.57	---	---
12986	AU02_01	Leona River at Loma Vista Road (CR 4757)	28.840500	-99.407627	1.76	59.71	31.33	Public	---
---	AU02_02	Leona River upstream of the crossing of Loma Vista Road (CR 4757)	28.838630	-99.412623	0.36	59.35	31.69	Private	Yes
---	AU02_03	Leona River about 7 river miles downstream of the crossing with FM 1866	28.863979	-99.536055	12.82	46.53	44.51	Private	Yes
---	AU02_04	Leona River below the confluence of Gallina Slough	28.869597	-99.553249	1.86	44.67	46.37	Private	Yes
21064	AU02_05	Leona River on FM 1866 above the confluence of Gallina Slough	28.905851	-99.577419	5.06	39.61	51.43	Public	---
---	AU02_06	Leona River in Batesville off of Ramos Street	28.953705	-99.609648	6.65	32.96	58.08	Private	Yes

TCEQ ID (if collocated)	Site ID	Site Description	Latitude (NAD83)	Longitude (NAD83)	Estimated Distance to Previous Station (miles) ¹	Estimated Distance from Upper Segment Boundary (miles) ¹	Estimated Distance from Lower Segment Boundary (miles) ¹	Private or Public Access	Private Access Landowner Approved
---	AU02_07	Leona River within Batesville City Park	28.961607	-99.61863	0.70	32.26	58.78	Public	---
12987	AU02_08	Leona River at US 57 near Batesville	28.963631	-99.614258	0.17	32.09	58.95	Public	---
---	AU02_09	Leona River about 1.3 river miles upstream of the crossing of US 57	28.978310	-99.613280	1.27	30.82	60.22	Private	Yes
---	AU02_10	Leona River near dam within Batesville City Park	28.983649	-99.618600	0.91	29.91	61.13	Public	---
*2	AU02_11	Leona River at low water crossing off CR 1005B; just below confluence of Camp Lake Slough	29.010794	-- 99.621517	2.51	27.40	63.64	Public	---
---	---	[AU02 upper / AU03 lower boundary at confluence of Camp Lake Slough]	---	---	0.80	27.29	63.75	---	---
---	AU03_01	Leona River about 0.9 river miles upstream of CR 1005B	29.021247	-99.623040	0.11	26.49	64.55	Private	Yes

2 TCEQ station 21066 is within 300 meters upstream of AU02_11, but the two sites are not considered collocated, because station 21066 is located above the confluence of Camp Lake Slough and the crossing of CR 1005B is below the confluence of Camp Lake Slough. TCEQ station 21066 is not considered as a separate RUAA site, because of the close proximity of RUAA site AU03_01. RUAA site AU03_01 (above the confluence with Camp Lake Slough) is within a mile of this location.

TCEQ ID (if collocated)	Site ID	Site Description	Latitude (NAD83)	Longitude (NAD83)	Estimated Distance to Previous Station (miles) ¹	Estimated Distance from Upper Segment Boundary (miles) ¹	Estimated Distance from Lower Segment Boundary (miles) ¹	Private or Public Access	Private Access Landowner Approved
---	AU03_02	Leona River about 4.4 river miles upstream of CR 1005B3	29.060589	-99.634597	3.53	22.96	68.08	Private	Yes
---	AU03_03	Leona River about 5.4 river miles upstream of CR 1005B	29.067696	-99.646576	1.00	21.96	69.08	Private	Yes
---	AU03_04	Leona River about 3 river miles downstream of USGS gaging station 08204005	29.133371	-99.709184	8.17	13.79	77.25	Private	Yes
12988	AU03_05	Leona River SE of Uvalde at USGS gaging station 08204005	29.153347	-99.740833	3.09	10.70	80.34	Private	Yes
12989	AU03_06	Leona River at Hoags Dam on Fort Inge, below confluence of Cooks Slough	29.170088	-99.763183	2.00	8.70	82.34	Limited Public ⁴	Yes
---	AU03_07	Leona River on Fort Inge above the dam and above the confluence with Cooks Slough	29.178120	-99.766815	0.66	8.04	83.00	Limited Public ⁴	Yes
12990	AU03_08	Leona River at FM 140	29.188787	-99.770980	1.01	7.03	84.01	Public	---
12992	AU03_09	Leona River at US 90 West in Uvalde	29.211790	-99.779766	2.27	4.76	86.28	Public	---

3 The location of RUAA site AU03_02 was estimated from maps based on a description from the landowner and not based on GPS coordinates collected at the site due to access issues associated with road conditions on the private land at the time reconnaissance was completed.

4 Fort Inge is open only on weekends from 8 a.m. to 8 p.m. and on special occasions for public access.

TCEQ ID (if collocated)	Site ID	Site Description	Latitude (NAD83)	Longitude (NAD83)	Estimated Distance to Previous Station (miles) ¹	Estimated Distance from Upper Segment Boundary (miles) ¹	Estimated Distance from Lower Segment Boundary (miles) ¹	Private or Public Access	Private Access Landowner Approved
---	AU03_10	Leona River within Uvalde off Leona St	29.217066	-99.783475	0.43	4.33	86.71	Public	---
---	AU03_11	Leona River within Uvalde off Studer St	29.22523	-99.784254	0.59	3.74	87.30	Public	---
---	AU03_12	Leona River within Uvalde off Rio St	29.228436	-99.788050	0.47	3.27	87.77	Private	Yes
---	AU03_13	Leona River at crossing of FM 2369	29.235905	-99.782799	0.61	2.66	88.38	Public	---
---	AU03_14	Leona River at US 83 north of Uvalde [SEGMENT & AU03 upper boundary at US 83]	29.256881	-99.778394	2.66	0.00	91.04	Public	---

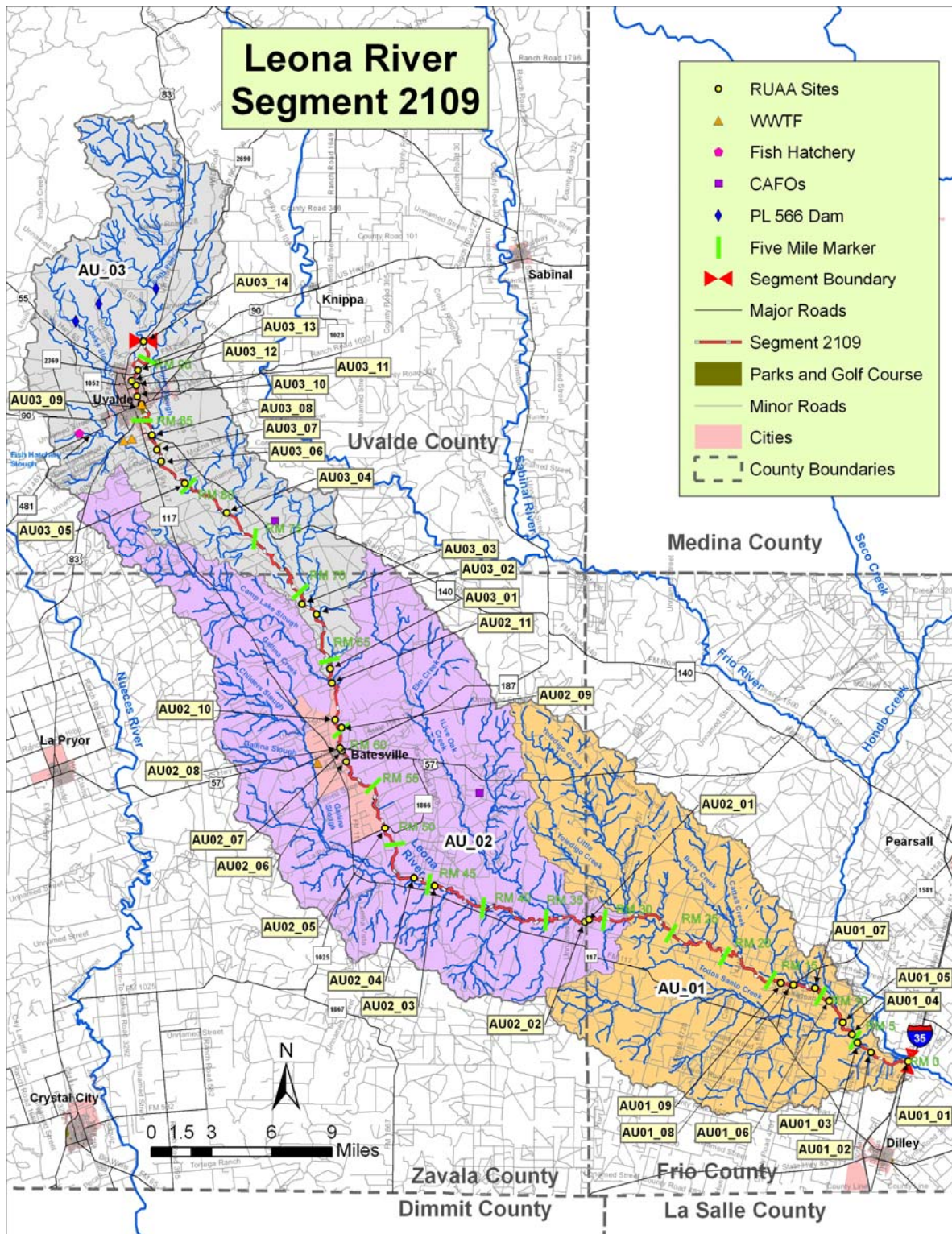


Figure B1.2. Map of RUAA sites along the Leona River.

B2 Sampling Methods

Field Sampling Procedures

Field sampling and measurements will be conducted according to procedures documented in the *TCEQ SWQM Procedures Volume 1: Physical and Chemical Monitoring Methods, (RG-415)* most recent edition and updates issued by TCEQ.

Field parameters will be measured during all routine sampling events at all stations. Field parameters for temperature, specific conductance, pH and DO will be obtained using a YSI Model 600XLM multi-parameter sonde. Flow severity estimates will be documented and flow measurements will be taken during each sampling event. Flow measurements will be conducted using a SonTek FlowTracker or other appropriate equipment or method as dictated by water levels and equipment availability. For stations collocated with USGS gauging stations, information from the USGS gauging station will be used in lieu of direct flow measurements by TIAER. For permitted discharge stations, information from the permitted facility may be used in lieu of direct flow measurements if circumstances in the field prevent direct measurement. For springs or wells, depending on conditions, direct flow measurements may not be possible and the reason for this shall be reported on the field data sheet comments. While it is preferable to sample flowing waters, pools will be sampled if flowing water is not present, if the pool is sufficiently deep for a sample to be collected without contamination. If conditions at ambient stream sites are pooled (whether a sample is collected or not), then zero flow should be documented and a flow severity of No Flow (1). If the stream is dry, a flow severity of Dry (6) should be recorded and reported. When the stream is dry no record is reported for instantaneous flow (parameter 00061). Flow measurements will be made following the guidelines outlined in the *TCEQ SWQM Procedures Volume 1: Physical and Chemical Monitoring Methods (RG-415)* and manufacturer's instructions.

Continuous water level recorders will be installed at three mainstem stations (See Table B1.1 for station IDs). Flow measurements will be made during each monitoring event in order to develop and refine a rating curve for each station. Cross sectional areas and/or Manning's equation may also be used in the development of the rating curve. The stage indicated by the flow meter during each event will be recorded onto the field data sheet. After the rating curve is developed, the stage reported on the field sheets will be converted to discharge. The rating curve development process may take multiple months to complete depending upon the amount of rainfall received in the watershed and subsequent runoff events. Additional flow measurements beyond those collected during routine monitoring at stream gauging sites will be obtained on an opportunistic basis throughout the project in an effort to reflect a variety of water levels in development of the stage-discharge relationship at each site.

During sampling events, field data sheets will be completed for each sampling station, regardless of flow status. The section "Documentation of Field Sampling Activities Data" (below) lists the data to be recorded at each station.

Water samples will be collected directly from the stream at mid-channel into containers as specified in Table B2.1. Routine samples for total nitrite-nitrate nitrogen are collected in a clean plastic bottle. The bottle will be agitated thoroughly to ensure total mixing of sediments that may have settled, then poured

into an acidified container that is capped and shaken to disperse the acid throughout the sample.

All bacteria samples will be collected mid-channel and upstream of bridge and road crossings. The bacteria samples will be collected at 0.3 meter depth or at mid-depth if the stream or outfall is less than 0.3 meter deep directly into the sample bottle. At sites where samples are collected by the technician entering the stream, the sample will be collected upstream of the technician and away from disturbed sediments. Bacteria samples are collected in sterile, disposable plastic 290 mL bottles that have been factory autoclaved and sealed and include sodium thiosulfate to neutralize up to 15 mg/L of chlorine residual samples for bacteria will be screened in the laboratory for the presence of chlorine residual for *E. coli* mTec. For *Bacteroidales*, new sterile bottles will be used without sodium thiosulfate. Bacteria samples are labeled as outlined in Section B3, iced immediately in the field, and transported to the laboratory.

Fecal samples for known source analysis will be obtained in one of four ways: 1) collecting fecal samples from areas where animals were visually observed defecating by technician; i.e. deer or feral hogs at feeders; 2) gut samples collected from animals recently killed by cars (within 24 hours), 3) legally harvested by hunters who have agreed to work with the technician, or 4) human (wastewater) samples collected from septic tanks or from influent (pre-secondary treatment) at wastewater treatment plants. Alternatively, fecal samples representing human waste may be collected from individual people. Gut samples will be collected by using sterile loops inserted anally or by cutting into the intestine using a sterile scalpel. Fecal samples will be placed in a fecal tube and refrigerated or kept on ice prior to shipping to SCSC as per SOP in Appendix D.

Container types, expected sample volumes, preservation requirements, and holding time requirements are specified in Table B2.1.

Table B2.1 Sample Storage, Preservation and Handling Requirements

Parameter	Matrix	Container	Field Preservation or Handling	Sample Volume	Holding Time
Total Nitrite + Nitrate-Nitrogen	Water	Pre-cleaned plastic	pH<2 with H ₂ SO ₄ ; cool to <6°C	60 mL	28 days
<i>E. coli</i> , mTec	Water	Sterile plastic	Sodium thiosulfate added; cool to 4°C	250 mL	8 hours
Fecal specimen	Feces	Sterile Container	Cool to 4°C	30 g	5 days
<i>E. coli</i> water isolates from <i>E. coli</i> mTec	Water	Petri dish 50mm x 9mm	Ice/refrigeration, cool to 4°C	See <i>E. coli</i> , mTec	24 – 48 hrs, then shipped to SCSC
<i>Bacteroidales</i>	Water	New Sterile container	Ice/refrigeration, cool to 4°C	100 mL	6 hrs for filtration; filters indefinitely

The RUAA surveys will be based on the *TCEQ Procedures for a Comprehensive Recreational UAA and a Basic UAA Survey*. Field measurements will be made according to procedures documented in the *TCEQ SWQM Procedures Volume 1: Physical and Chemical Monitoring Methods, 2012 (RG-415)*. Water temperature will be measured using calibrated YSI 600 XLM multiprobes. Air temperature will be measured using hand-held field thermometers. Instantaneous water velocity measurements (flow) will be measured using SonTek Flow Tracker™ Acoustic Doppler Velocimeter. TIAER personnel will use the streamflow measurement form developed by TIAER.

For the RUAA field surveys, information to be collected shall at least satisfy those questions found on the Field Data Sheet in Appendix G.

Processes to Prevent Cross Contamination

Procedures outlined in the *TCEQ SWQM Procedures Volume 1* outline the necessary steps to prevent cross-contamination of samples. These include such things as direct collection into sample containers and the use of commercially pre-cleaned sample containers.

Documentation of Field Sampling Activities

Field sampling activities associated with routine water quality monitoring are documented on the Field Data Sheet as presented in Appendix A. For all visits where water quality samples are collected, station ID, location, sampling time, sampling date, sampling depth, preservatives added to samples, and sample collector's name/signature are recorded. Values for all measured field parameters are recorded. Detailed observational data are recorded including water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, missing parameters, days since last significant rainfall, and flow severity. Conditions permitting, photos upstream, downstream, right bank and left bank will also be recorded for each site during each sampling period to document stream conditions.

The following will be recorded for all visits:

1. Station ID
2. Sampling Date
3. Station Description
4. Sampling depth
5. Sampling time
6. Sample collector's name/signature
7. Values for all field parameters
8. Detailed observational data, including:
 - a. water appearance
 - b. weather
 - c. biological activity
 - d. unusual odors
 - e. pertinent observations related to water quality or stream uses
 - f. watershed or instream activities
 - g. specific sample information
 - h. missing parameters

With regard to fecal samples for known source analysis, the following information will be reported as per Appendix D3: Collection of Fecal Samples for Bacterial Source Tracking, using the Known Source COC sheet in Appendix B:

- a. Sampling date
- b. Animal species
- c. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
- d. Sample collector's name/initials
- e. Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle

RUAA survey activities are documented on the Field Data Sheet, Interview Forms, and RUAA Summary Sheet as found in the 2009 *TCEQ Procedures for a Comprehensive RUAA and a Basic RUAA Survey*.

Recording Data

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

- 1 Legible writing in indelible, waterproof ink with no modifications, write-overs or cross-outs;
- 2 Changes should be made by crossing out original entries with a single line, entering the changes, and initialing and dating the corrections.
- 3 Close-outs on incomplete pages with an initialed and dated diagonal line.

Deficiencies, Nonconformances and Corrective Action Related to Sampling Requirements

Deficiencies are defined as unauthorized deviations from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render data unacceptable or indeterminate. Deficiencies related to sampling method requirements include, but are not limited to, such things as sample container, volume, and preservation variations, improper/inadequate storage temperature, holding time exceedances, and sample site adjustments.

For TIAER, deficiencies in field sampling activities are documented in logbooks and field data sheets by field or laboratory staff and reported via CAR to the pertinent field or laboratory manager. The supervisor will forward the CAR to the QAO. If the situation requires an immediate decision concerning data quality or quantity, the TIAER Project Manager (or designee) will be notified within 24 hours. The TIAER Project Manager (or designee) will notify the TIAER QAO of the potential nonconformance. The TIAER QAO will record and track the CAR to document the deficiency.

The TIAER QAO, in consultation as appropriate with the TIAER Project Manager (and other affected individuals/organizations), will determine if the deficiency constitutes a nonconformance. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TIAER Project Manager in consultation with TIAER QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TIAER QAO.

CARs document: root cause(s); programmatic impact(s); specific corrective action(s) to address the deficiency; action(s) 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. The TSSWCB will be notified of excursions that affect data quality with QPRs. In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to the TSSWCB immediately.

B3 Sample Handling and Custody

Sample Labeling

Water samples will be labeled on the container with an indelible, waterproof marker. Label information includes:

1. Sample Number, Bottle Letter, and Site Number
2. Date and time of collection
3. Sample Depth
4. Initials of collector

A TIAER COC form will accompany all sets of sample containers.

For the Known Source fecal samples will include at a minimum the following label information:

- a. Sampling date
- b. Animal species
- c. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
- d. Sample collector's name/initials

A Known Source Fecal Sample COC will accompany all sets of fecal samples.

Water Quality Sample Handling

All samples are collected according to TCEQ SWQM procedures. All water samples are iced in the field and submitted to the TIAER mobile laboratory on ice the same day they are collected in the field. After samples are received at the laboratory, they are inventoried against the accompanying COC. Any discrepancies are noted at that time, remediated if possible, and the COC is signed for acceptance of custody. Sample numbers are assigned, and samples are checked for preservation (as allowed by the specific analytical procedure). Samples are then filtered or pretreated as necessary and placed in a refrigerated cooler dedicated to sample storage, as required.

The laboratory manager has the responsibility to ensure that all holding times are met (see Tables B2.1 and B2.2). Any problems will be documented with a CAR.

Known Source Fecal Sample Handling

Fecal samples will be placed in a fecal tube and refrigerated or kept on ice prior to shipping to SCSC as per SOP in Appendix D.

BST Sample Handling

All samples used in BST analysis will be collected and prepared by TIAER prior to shipment to SCSC. Preprocessing of BST samples will follow SOPs provided by SCSC for library-independent samples (*Bacteroidales* PCR) and library-dependent samples (Isolation of *E. coli* from Water Samples) as provided in Appendix D. TIAER will periodically ship or arrange to deliver bacterial cultures and/or *Bacteroidales* filters following shipping procedures outlined in Appendix D to SCSC for BST analyses.

TIAER will receive water samples and preprocess them for *E. coli* isolation for library-dependent BST samples. *E. coli* will be isolated from the water samples using USEPA Method 1603 and modified membrane Thermotolerant *E. coli* (mTEC) medium. Inoculated plates will be incubated at 35±0.5°C for 2

hours to resuscitate stressed bacteria, and then incubated at $44.5 \pm 0.2^\circ\text{C}$ for approximately 20 to 24 hours. After pre-processing and enumeration by TIAER lab personnel, the plates will be shipped to SCSC, which will transfer *E. coli* colonies from the modified mTEC medium onto nutrient agar with MUG to confirm glucuronidase activity and culture purity. The *E. coli* plates will be shipped to SCSC in insulated coolers with sufficient ice to maintain about 4°C .

Additionally, the TIAER lab will also preprocess water samples for *Bacteroidales* PCR analysis for library-independent samples. TIAER will preprocess water samples for *Bacteroidales* PCR by passing ~100 ml (4 aliquots x 25 ml) of each water sample through $0.2\ \mu\text{m}$ filters (a separate 25 ml aliquot through each of four separate filters per water sample) to collect biomass and then freezing each filter until shipment to SCSC for analysis. Filters should be shipped in insulated coolers with dry ice sufficient to keep samples frozen during transit.

Sample Tracking

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. A sample is in custody if it is in actual physical possession or in a secured area that is restricted to authorized personnel. The COC form documents possession of samples from the time of collection to receipt in the laboratory. The following information is recorded on the TIAER COC form for water samples (See Appendix B).

1. Date and time of collection
2. Site identification
3. Sample matrix
4. Number of containers
5. Residual chlorine
6. Preservative used
7. Was the sample filtered
8. Analyses required (indicated by test group code)
9. Name of collector
10. Custody transfer signatures and dates and time of transfer

For Known Source fecal samples the following information is recorded Known Source COC form (Appendix B):

1. Sampling date
2. Animal species
3. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
4. Sample collector's name/initials
5. Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle

Deficiencies, Nonconformances and Corrective Action Related to Sample Handling

Deficiencies related to sample handling are documented in logbooks and field data sheets by field or

laboratory staff and reported via CAR to the pertinent field or laboratory manager. At TIAER the appropriate supervisor will forward the CAR to the TIAER QAO. If the situation requires an immediate decision concerning data quality or quantity, the TIAER Project Manager (or designee) will be notified within 24 hours. The TIAER Project Manager (or designee) will notify the TIAER QAO of the potential nonconformance. The TIAER QAO will record and track the CAR to document the deficiency.

If a sampling handling deficiency is noted by SCSC for BST samples, the TIAER PM shall be notified and a CAR produced. The TIAER PM will notify the appropriate field staff member, manager of the TIAER or SCSC Laboratory, and the TIAER QAO about the sample handling CAR so it may be recorded and tracked.

The TIAER QAO, in consultation as appropriate with the TIAER Project Manager (and other affected individuals/organizations), will determine if the deficiency constitutes a nonconformance. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TIAER Project Manager in consultation with TIAER QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TIAER QAO. The TSSWCB will be notified of excursions that affect data quality with QPRs. In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to the TSSWCB immediately.

B4 Analytical Methods

Table B4.1 presents the analytical equipment used for project analyses specified in Table A7.1.

Table B4.1. Laboratory and Field Analytical Methods and Equipment

Parameter	Method ¹	Equipment Used
Laboratory Parameters		
Nitrite-Nitrogen+Nitrate Nitrogen	SM online 4500-NO3 F-00	Lachat QuickChem Autoanalyzer
<i>Escherichia coli</i>	EPA 1603	Millipore incubator with battery
Field Parameters		
Dissolved Oxygen	EPA 360.1, TCEQ SOP, V1	YSI Multiprobe
Potential Hydrogen	EPA 150.1, TCEQ SOP, V1	YSI Multiprobe
Specific Conductance	EPA 120.1, TCEQ SOP, V1	YSI Multiprobe
Water Temperature	EPA 170.1, TCEQ SOP, V1	YSI Multiprobe
Instantaneous Flow	TCEQ SWQM	Global Water FlowProbe, Pygmy Flow Meter, Price Flow Meter, SonTek FlowTracker, or RDI- Acoustic Doppler Current Profiler
Automated Stream Level	TIAER SOPs F-110, F-111, & F-114	ISCO Flowmeter

¹ Some methods are modified by TIAER as outlined in Table A7.1.

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

SM = Standard Methods for Examination of Water and Wastewater, 18th or latest online editions

TCEQ SWQM = Texas Commission on Environmental Quality Surface Water Quality Monitoring Procedures, Volume 1 (RG-415, most recent version)

TIAER SOPs for operation of ISCO Flowmeter for automated stream level recording are presented in Appendix F.

Water Quality Analytical Methods

The analytical methods are listed in Table A7.1. Laboratories collecting data under this QAPP are compliant with the NELAP Standards, where applicable.

Copies of laboratory SOPs are retained by TIAER and are available for review by the TSSWCB. Laboratory SOPs are consistent with EPA requirements as specified in the method.

Standards Traceability

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

Deficiencies, Nonconformances and Corrective Action Related to Analytical Methods Performed by TIAER

Deficiencies related to analytical methods are noted by TIAER laboratory staff and reported via CAR to the laboratory manager and then forwarded to the QAO. If the situation requires an immediate decision concerning data quality or quantity, the TIAER Project Manager will be notified within 24 hours. The TIAER Project Manager will notify the TIAER QAO of the potential nonconformance. The TIAER QAO will record and track the CAR to document the deficiency.

The TIAER QAO, in consultation as appropriate with the TIAER Project Manager (and other affected individuals/organizations), will determine if the deficiency constitutes a nonconformance. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TIAER Project Manager in consultation with TIAER QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TIAER QAO. The TSSWCB will be notified of excursions that affect data quality with quarterly progress reports. In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to TSSWCB immediately.

BST Analyses

The analytical methods utilized in BST analysis and sample preparation are listed in Table A7.2 and described in detail in SCSC SOPs (Appendix D).

E. coli in water samples will be quantified and isolated by TIAER personnel using modified mTEC agar, EPA Method 1603 (USEPA 2006). Known source fecal samples will be isolated by SCSC also using EPA Method 1603. 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 SCSC and streaked for purity on nutrient agar with MUG to confirm glucuronidase activity and culture purity. Cultures of selected isolates will be archived using glycerol freezing medium (-80°C). Inoculated plates will be incubated at 35±0.5°C for 2 hours to resuscitate stressed bacteria, and then incubated at 44.5±0.2°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-RP SOPs. DNA patterns of those isolates will be compared to the Texas *E. coli* BST Library of *E. coli* isolates from known animal and human sources collected throughout Texas.

As outlined in Appendix D, 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 SCSC 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.

Deficiencies, Nonconformances and Corrective Action Related to Analytical Methods Performed by SCSC

Failures in 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 lab analyst will be able to correct the problem. If the problem is resolvable by the 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 SCSC Project Lead, who will make the determination in coordination with the TIAER 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 SCSC project lead will include this information in the CAR (Appendix C) for submittal with the QPR, which is sent to the TSSWCB PM via the TIAER PM.

B5 Quality Control

Quality Control Requirements and Acceptability Criteria for Conventional Parameters

Table A7.1 lists the required accuracy, precision, and completeness limits for the conventional parameters of interest, nitrite+nitrate nitrogen and *E. coli*. It is the responsibility of the TIAER PM (or designee) to verify that the data are representative. All incidents requiring corrective action will be documented through use of CARs. Laboratory audits, sampling site audits, and QA of field sampling methods will be conducted by the TSSWCB QAO (or designee).

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 *TCEQ SWQM Procedures, Volume 1*. Split samples are preserved, handled, shipped, and analyzed identically and are used to assess variability in all of these processes. For this project, field splits are collected for 10 percent of total nitrite+nitrate nitrogen samples only (not bacteria).

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, then 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.

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 QAM. The minimum requirements that all participants abide by are stated below.

Limit of Quantitation (LOQ)

The TIAER laboratory will analyze a calibration standard (if applicable) at the LOQ on each day project samples are analyzed. Calibrations including the standard at the LOQ will meet the calibration requirements of the analytical method or corrective action will be implemented.

LOQ Check Standard

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.

As noted above, the LOQ check standard will be used for information in determining the performance of the measurement system at the lower limits of analysis and not as a sole criterion for determining overall data acceptability for a batch.

Laboratory Control Sample (LCS)

An LCS consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system. The LCS is spiked into the sample matrix at a level less than or near the 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 multipeak 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 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 as follows:

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

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are analyzed. 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 > 20 organisms/100mL.

Matrix spike

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 sample 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% for nitrite-nitrate nitrogen spikes, 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.

Deficiencies, Nonconformances and Corrective Action Related to Quality Control by TIAER

Deficiencies noted by TIAER are documented in logbooks and field data sheets by field or laboratory staff and reported via CAR to the pertinent field or laboratory manager. The supervisor will forward the CAR to the QAO. If the situation requires an immediate decision concerning data quality or quantity, the TIAER Project Manager will be notified within 24 hours. The TIAER Project Manager will notify the TIAER QAO of the potential nonconformance. The TIAER QAO will record and track the CAR to document the deficiency.

The TIAER QAO, in consultation as appropriate with the TIAER Project Manager (and other affected individuals/organizations), will determine if the deficiency constitutes a nonconformance. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TIAER Project Manager in consultation with TIAER QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TIAER QAO. The TSSWCB will be notified of excursions that affect data quality with QPRs. In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to TSSWCB immediately.

Quality Control Requirements and Acceptability Criteria for BST Parameters

Table A7.2 lists the required accuracy, precision, and completeness limits for the BST 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 C). 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 SCSC for each batch of *E. coli* ERIC-RP, 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 A7.2.

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:

$$RPD = [(X_1 - X_2) \times 100] / [(X_1 + X_2) \div 2]$$

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 are used to determine the acceptability of duplicate analyses. Precision limits for bacteriological analyses are defined in Table A7.2 and applies to samples with concentrations >20 cfu/100 ml.

Failures in Quality Control and Corrective Action

Notations of blank contamination will be noted in QPRs and the final BST 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 PMs and QAOs. The SCSC project lead will include this information in the CAR for submittal with the QPR, which is sent to the TSSWCB PM via the TIAER PM.

B6 Instrument/Equipment Testing, Inspection and Maintenance

All equipment inspection and maintenance requirements for project activities will follow manufacturer and annual preventative maintenance guidance for each instrument and equipment item.

Surface Water Quality Monitoring

All sampling equipment testing and maintenance requirements are detailed in the latest version of and updates to *TCEQ Surface Water Quality Monitoring Procedures (Volume 1)* and TIAER SOPs for flowmeter initialization and operation (Appendix F). 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 SOPs.

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 TIAER QAO.

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, latest online 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 via TIAER. The CARs will be maintained by the TIAER QAO.

RUAA

All sampling equipment testing and maintenance requirements are detailed in the TCEQ Surface Water Quality Monitoring Procedures, Volume 1. Field equipment is inspected and tested upon receipt and is assured appropriate for use. Acceptance criteria are detailed in the TIAER's quality assurance manual. Equipment records are kept on all field equipment and are available for inspection by the TSSWCB. A supply of critical spare parts is maintained by the TIAER Field Supervisor, or designee.

B7 Instrument/Equipment Calibration and Frequency

Equipment inspection and maintenance requirements for all equipment used for project activities are performed according to manufacturer and annual preventive maintenance guidance for each instrument and equipment item.

Surface Water Quality Monitoring

Field equipment calibration requirements are contained in the latest version of and updates to the *TCEQ Surface Water Quality Monitoring Procedures* and TIAER SOPs for flowmeter initialization and programming (Appendix F). 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. Detailed laboratory calibrations are contained within the TIAER QAM.

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 PCR Thermal cycler and RiboPrinter instruments will be calibrated per Product Owner's Manual. 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 SCSC SOPs are available for review upon request. 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 via TIAER. The CARs will be maintained by the SCSC Project Leader.

RUAA

Field equipment calibration requirements are contained in the latest version of and updates to the *TCEQ Surface Water Quality Monitoring Procedures*.

B8 Inspection/Acceptance of Supplies and Consumables

Water Quality Analysis

New batches of TIAER supplies are tested and the results recorded in the appropriate logbook before use to verify that they are not contaminated. The TIAER QAM provides additional details on acceptance requirements for laboratory supplies and consumables.

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.

RUAA

All new batches of field supplies are inspected before use to ensure that they are adequate. Acceptance criteria are detailed in TIAER’s SOP-Q-102, Laboratory Material Acceptance Criteria.

B9 Non-Direct Measurements

TIAER will coordinate with CRP monitoring within the Leona River watershed conducted by the NRA and TCEQ to avoid duplicative sampling. Data collected by the NRA and TCEQ will be considered acceptable for this project in that it requires similar QA for conventional and field parameters and is collected under a TCEQ approved QAPP.

In addition, flow and stage data from USGS gauging stations within the watershed may be used to determine flows at collocated sampling stations. Discharge information may also be obtained from permitted discharge facilities in lieu of direct flow measurements.

TIAER will conduct a historical data review for the waterbody in order to assess and characterize trends and variability in water quality, specifically bacteria and nitrite-nitrate nitrogen. The historical data collection activities will focus on ambient water quality data, including groundwater, streamflow and water level data, precipitation records, and permitted facilities, discharges, and effluent quality. Data sources will include U.S. Geological Survey, National Weather Service, Texas Parks and Wildlife Department, Texas Water Development Board, Groundwater Conservation Districts, Edwards Aquifer Authority, NRA, TCEQ, and the U.S. Environmental Protection Agency.

The existing Texas BST library maintained by the Texas Water Resources Institute and Texas AgriLife Research will be used as a resource and added to as part of the project. The web address is <http://twri.tamu.edu/programs/texas-bst-library>.

All other data for the project will be generated during the project according to requirements in this QAPP.

B10 Data Management

Data Path – TIAER Surface Water Quality Monitoring Data

Water quality samples are collected and transferred from the field to the laboratory for analyses as described in Section B3 using a TIAER COC form (Appendix B) following procedures in TIAER SOP-Q-110, Sample Receipt and Log In. A unique sample identification number is given to each sample at log in. Identifying sample information and comments are manually entered into the initial database queue. Laboratory measurement results are entered into a secondary database queue, either automatically or manually, depending on the instrument. Following laboratory data verification and validation, the data are transferred from the secondary queue database to the master queue within the TIAER LIMS. At this point, any additional manually generated field data or comments are added to the LIMS database by the field crew and validated by a separate individual. Data from TIAER's LIMS are then uploaded to a SAS software database, which is used for statistical evaluation of the data to evaluate project objectives. Procedures and personnel involved in data entry and review are outlined in TIAER SOP-Q-104, Data Entry and Review.

Field parameters collected with the YSI multiprobe (pH, water temperature, conductivity, and dissolved oxygen) are automatically downloaded from the instrument and imported into an EXCEL spreadsheet. Printouts of the sonde data are compared with manually entered data on the field data sheets for validation. The electronic sonde data are then exported to a SAS database and automatically merged with the SAS database containing the LIMS data by site, date, and time and again reviewed by field crew personnel to make sure the data merge occurred correctly.

Stream water level data collected using ISCO flowmeter will be downloaded using field laptop computers routinely every two weeks, when routine sampling occurs, and stored in a SAS or WISKI database for review. Records of site visits to download the flow meters are kept on the GM sheets (Appendix A). Stream level data are reviewed in WISKI by appropriate field staff and then transferred back to SAS for storage.

Following data verification and validation, data appropriate for SWQMIS are exported from the database to pipe-delimited text files in TCEQ format for reporting to the TSSWCB. Upon completion of a data review, TSSWCB will submit these files, as appropriate, to TCEQ for entry into SWQMIS.

On a quarterly basis, all project data will be submitted to the TSSWCB for review in a format suitable for posting on the project website maintained by the NRA. All data posted on the project website will be flagged as preliminary and subject to change.

Data Path - SCSC

Once samples are received at SCSC, samples are logged and stored at 4°C or -80 °C (as appropriate) until processed. The COC will be checked for number of samples, proper and exact ID number, signatures, dates, and type of analysis specified. TIAER will be notified if any discrepancy is found and proper corrections made. The COC and accompanying 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 designee 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 SCSC. 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 five years after the end of the project.

Preliminary BST data will be transferred from the SCSC to the TSSWCB as needed to provide preliminary data at project meetings or prior to stakeholder presentations. Preliminary BST data in the form of the presentations on the BST work will also be posted on the project website with the TSSWCB approval. An official, technical report on the final BST results will be provided at the end of the study.

Data Path – TIAER RUAA Data

TIAER will complete Field Data Sheets for the Basic RUAA, Contact Information Forms, and Comprehensive RUAA Interview Forms by hand as hard copies or as electronic forms on a computer. Information entered by hand will be scanned and also entered into electronic versions of the forms at the TIAER office in a directory specifically designated for the project that is backed up incrementally every evening and completely once a week. A TIAER staff member other than the person who electronically entered the data will review at least 10 percent of the survey information in the database against the original hard copies. TIAER staff members will enter data electronically onto the RUAA Summary Sheet into the project directory. Photographs will be taken according to guidelines in the 2009 *Procedures for a Comprehensive RUAA and a Basic RUAA Survey*. The photographs will be taken by an electronic camera and stored in a jpg format in the project directory. These data will be submitted to the TSSWCB by the TIAER project manager or designee and are to accompany the draft of the final RUAA report when submitted to the TSSWCB.

Record-Keeping and Data Storage

TIAER record-keeping and document control procedures are contained in the TIAER Quality System Statement and this QAPP. Original field and laboratory data sheets are stored in the TIAER offices, laboratory, and storage facility in accordance with the record-retention schedule in Section A9. As an electronic data protection strategy, TIAER utilizes Double Take software to mirror the Primary Aberdeen 1.2TB file server (raid 5 fault tolerant) that will be mirrored to a secondary Aberdeen Abernas211 file server (raid 5 fault tolerant). This provides instant fault recovery rollover capability in the event of hardware failure. TIAER also exercises complete backup of its Primary server to LTO 3 Quantum ValueLoader on a weekly basis, coupled with daily incremental backups. This provides a third level of fault tolerance in the event that both the primary and secondary servers are disabled. TIAER will maintain all cyclic back-up tapes for 26 weeks prior to reuse saving the 1st tape in the series indefinitely to preserve an historical snapshot. This will facilitate recovery of data lost due to human error. Backup tapes are stored in a secure area on the Tarleton State University campus and are checked periodically to

ensure viability. If necessary, disaster recovery can also be accomplished by manually re-entering the data.

SCSC maintains all data sheets and laboratory notebooks for at least five years. Data spreadsheets are maintained on the computer's hard drive, which is also simultaneously saved in a network folder and backed up on a weekly basis.

Data Verification/Validation

The control mechanisms for detecting and correcting errors and for preventing loss of data during data reduction, data reporting, and data entry are contained in Sections D1, D2, and D3.

TIAER laboratory technicians review all data before finalizing data. The Laboratory Manager reviews all data following analysis and checks for calculation errors or data entry errors. The TIAER LQAO performs a third review of data to determine validity within this QAPP.

The SCSC Project Lead or designee reviews all manually entered data for accuracy to ensure that there are no transcription errors.

Data that are not valid, for quality reasons, will not be submitted to the TSSWCB. This determination will be made by the TIAER PM/QAO and/or SCSC Project Leader in coordination with the TSSWCB PM and QAO.

Forms and Checklists

See Appendix A for the Field Data Sheets and Appendix E for the Data Summary Checklist.

Data Handling, Hardware, and Software Requirements

For data handling, TIAER utilizes standard, IBM compatible, desktop personal computers that utilize a MS Windows operating system. TIAER utilizes MS Access 2007 as the primary database management software. TIAER's Water Quality Database has been developed according to CRP guidance and database structures in accordance with TSSWCB and TCEQ requirements. Hardware configurations are sufficient to run Microsoft Access and SAS software in a networked environment. Specific hardware is also configured to run WISKI and FLOWLINK software, but not necessarily in a networked environment for continuous stage data. TIAER information resources staff is responsible for assuring that hardware configurations meet the requirements for running current and future data management/database software as well as providing technical support.

The SCSC laboratory uses electronic spreadsheets created in Microsoft© Excel software for data handling on an IBM-compatible microcomputer with a Windows® operating system.

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 Requirements

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TIAER and SCSC Project Managers	Monitor project status and records to ensure requirements are being fulfilled.	Report to TSSWCB in QPRs
Laboratory Inspection	At least once during the project period.	TSSWCB	Analytical and quality control procedures employed at the laboratories	45 days to respond in writing to TSSWCB to address corrective actions
Technical Systems Audit	At least once during the project period.	TSSWCB	Assess compliance with QAPP; review facility and data management as they relate to the project	45 days to respond in writing to TSSWCB to address corrective actions
Monitoring Systems Audit	At least once during the project period.	TSSWCB	Assess compliance with QAPP; review field sampling, facility and data management as they relate to the project	45 days to respond in writing to TSSWCB to address 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 shows 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 no less than once over the contractual period of the project. 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 TIAER 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, *TCEQ SWQM Procedures*, TIAER or SCSC SOPs. Deficiencies may invalidate resulting data and may require corrective action. Corrective action may include for samples to be discarded and recollected. Deficiencies are documented in logbooks, field data sheets, etc. by field or laboratory staff. It is the responsibility of each respective entity's PM, in

consultation with the TIAER 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 QPRs 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, 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 QPRs. 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 PM or Project Lead of 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 QPRs.

C2 Reports to Management

Reports to TSSWCB Project Management

All reports detailed in this section are contract deliverables and are transferred to the TSSWCB in accordance with contract requirements.

Quarterly Progress Reports – Summarize project activities for each task; reports problems, delays, and corrective actions; and outlines the status of each task’s deliverables. QPRs will be submitted by TIAER with input provided by each project entity.

Task 3 and 5 Reports – Summarize major tasks and include the following in association with the monitoring tasks outlined in this QAPP:

- Technical report detailing BST results developed by the SCSC Project Lead
- Technical report characterizing trends and variability in historical water quality monitoring data developed by TIAER
- Technical report characterizing trends and variability in collected water quality monitoring data developed by TIAER

(Note: Task 6 will be addressed in an amendment to the QAPP).

Task 6 Reports – Summarize information collected in association with the RUAA surveys and includes the following:

- Electronic copy of the Contact Information Form,
- Electronic copies of RUAA Field Data Sheets and the Data Summary Form
- Digital photographic record, cataloged in an appropriate manner,
- Electronic copies of Interview Forms and Interview Summary,
- Technical Report summarizing historical information review, field

All forms are to be from the 2009 version of the *TCEQ Procedures for a Comprehensive RUAA and Basic RUAA Survey*. Photographs will be organized and labeled in appropriate manner with guidance from the TSSWCB project manager, including provisions for providing multi-photo pdfs as well as individual jpg files.

D1 Data Review, Verification, and Validation

For the purposes of this document, data verification is a systematic process for evaluating performance and compliance of a set of data to ascertain its completeness, correctness, and consistency using the methods and criteria defined in the TIAER QAM, TIAER and SCSC SOPs, and this QAPP. Validation means those processes taken independently of the data-generation processes to evaluate the technical usability of the verified data with respect to the planned objectives or intention of the project. Additionally, validation provides a level of overall confidence in the reporting of the data based on the methods used.

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 that are supported by appropriate QC data and meet the measurement performance specification defined for this project will be considered acceptable and used in the project.

The procedures for verification and validation of data are described in Section D2. The TIAER PM is responsible for ensuring that field data are properly reviewed and verified for integrity. The TIAER Laboratory Supervisor is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and bias, and reviewed for integrity. The TIAER QAO is responsible for ensuring that all data are properly reviewed and verified, and submitted in the required format to the project database. The TIAER Laboratory QAO is responsible for validating a minimum of 10% of the laboratory data produced in each task. The SCSC Lab Director is responsible for ensuring that BST laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. Finally, the TIAER Project Manager, with the concurrence of the TIAER QAO, is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

D2 Verification and Validation Methods

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7 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 staff are listed in the first two sections of Table D2.1, respectively. 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 higher level project management to establish the appropriate course of action, or the data associated with the issue are rejected. Field and laboratory reviews, verifications, and validations are documented.

After the field and laboratory data are reviewed, another level of review is performed after the data are combined into a data set. This review step as specified in Table D2.1 is performed by the TIAER Data Manager and TIAER 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.

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 TIAER Project Manager validates that the data meet the DQOs of the project and are suitable for reporting to TSSWCB.

If any requirements or specifications 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 TIAER Data Manager with the data. This information is communicated to the TSSWCB by TIAER in the Data Summary.

Table D2.1: Data Review Tasks

Staff: PM – Project Manager; QAO – Quality Assurance Officer

Field Data Review	Responsibility
Field data reviewed for conformance with data collection, sample handling and COC, analytical and QC requirements	TIAER Field Supervisor
Post-calibrations checked to ensure compliance with error limits	TIAER Field Supervisor
Field data calculated, reduced, and transcribed correctly	TIAER Field Supervisor
Laboratory Data Review	Responsibility
Laboratory data reviewed for conformance with data collection, sample handling and COC, analytical and QC requirements to include documentation, holding times, sample receipt, sample preparation, sample analysis, project and program QC results, and reporting	TIAER or SCSC Laboratory Manager and QAO
Laboratory data calculated, reduced, and transcribed correctly	TIAER or SCSC Laboratory Manager and QAO
LOQs consistent with requirements for AWRLs	TIAER Laboratory Manager and QAO
Analytical data documentation evaluated for consistency, reasonableness and/or improper practices	TIAER or SCSC Laboratory Manager and QAO
Analytical QC information evaluated to determine impact on individual analyses	TIAER or SCSC Laboratory Manager and QAO
All laboratory samples analyzed for all parameters	TIAER or SCSC Laboratory Manager and QAO
Data Set Review	Responsibility
The test report has all required information as described in Section A9 of the QAPP	TIAER QAO and PM
Confirmation that field and laboratory data have been reviewed	TIAER QAO and PM
Data set (to include field and laboratory data) evaluated for reasonableness and if corollary data agree	TIAER QAO and PM
Outliers confirmed and documented	TIAER QAO and PM
Field QC acceptable (e.g., field splits and trip, field and equipment blanks)	TIAER QAO and PM
Sampling and analytical data gaps checked and documented	TIAER QAO and PM
Verification and validation confirmed. Data meets conditions of end use and are reportable	TIAER QAO and PM

D3 Reconciliation with User Requirements

Data produced in this project, and data collected by other organizations (e.g., USGS, TCEQ, etc.), will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be used by TSSWCB and other project partners to assess sources of bacteria through data analysis and modeling and to ascertain the suitability of the streams for contact recreation use in order to facilitate local decision-making. Since BST is an evolving science and no EPA-approved protocols currently exist, a discussion of the uncertainties surrounding source identification and the appropriate use of BST results will be included in the project final report. Additionally, data meeting project requirements will be submitted by the TSSWCB to the TCEQ for use in the biennial CWA §305(b) assessment for the *Texas Integrated Report*. Data that do not meet requirements will not be submitted to SWQMIS nor will it be considered appropriate for any of the uses noted above.

Appendix A

Example Field Data Sheets

General Maintenance

SITE _____ **DATE** _____ **TIME (CST)** _____ **INITIALS** _____

Level _____ **Enable** _____ **callout** _____

Battery ____% **New En/Dis** _____

Desiccants: OK Changed

Bottles: Full of Clean Needs _____ Added

Flowmeter SPA652 4230 3230

Sampler :

Display _____ **Reset to SI** Yes No
Reset arm to bottle 1 Yes
Checked distributor arm nut

Time interval: Uniform _____ **Reset start time** Yes Time _____
 NonUniform _____ **Reset start time** No

Sampling interval: Time Flow

Line: OK Clear Damaged Silted/Clogged
 Purged Acid Washed Test sample collected (monthly)
 Position in arm OK Reset

Pump tubing Current counts _____ Alarm counts _____
 Changed Reversed Checked all connections
 Reset counter # counts _____ Restart sampler YES

Bubbler: XS OK Silted Scoured Requires new survey
 Line OK Clear Damaged Requires new survey

TB Rain Gauge: Clear Cleaned Weekly inches recorded _____
 Checked operation Number of tips _____

QA rain gauge: Clear Cleaned Weekly inches collected _____

Downloaded: Sampler Flowmeter Met Viewed graph

Color Code: _____

Bottles used for composite: _____

Samples Missed: Yes No CAR number _____

Comments:

Field Data Sheet
Leona Grabs
 (Working draft: 20May2011)

Site:	TG Code:	Investigators:
Date:	Location:	Project:
Time	<i>run</i> <i>glide</i> <i>riffle</i> <i>pool</i>	General Climatological Observations

Hydrological Parameters

Total Depth: _____ ft.

Sample #	Sample Depth (ft)	Temp C	Conductus	DO mg/L	pH			Flow Sev. (select from below)
	*							
	1.00 **							
	record depth	* If total depth is <1.5 ft. collect at 1/3 total depth. ** If total depth >1.5 ft. collect at 1 ft.						
		Bacteria Sample - S (sterilized bottle)						
		tNO23N - D (acidified 250ml)						
		Field Split of Sample _____ Nitrate						

Estimated Flow Severity 1. no flow 2. low 3. normal 5. high 4. flood 6. dry
 Last Significant Rainfall (in days) <1 (w/in 24 hrs) 1 2 3 4 5 6 7 . . . _____

Datasonde used: _____

Comments:

Appendix B

Example Chain of Custody Forms for TIAER and Known Source Fecal Samples

TIAER										CHAIN OF CUSTODY				Page ___ of ___ Has field data? (Y/N)			
Project Manager/Person(s) Requesting Sample										Sampler(s)							
Project Code	Sample No.	Test Group Code	Sample Date(s) (mm/dd/yy)	Sample Time(s) (hr:mm)	CST for TIAER	Site ID	Sample Type	Matrix	Preservative	Container type/ number	Bottle information (if applicable)			Other comments:	pH check	temp check	Disposed
											bottle numbers:	start/end time/date:	composite: last bottle collection datetime				

Relinquished by: _____				Date/Time: _____		Received by: _____				Date/Time: _____	
Relinquished by: _____				Date/Time: _____		Received by: _____				Date/Time: _____	

Sample Types: G=Grab, SG=Storm Grab, S=Sequential, T=Time based, F=Flow based, M=Multisonde, P=Periphytin, O=other Matrix: L=Liquid, S=Solid										COC review initials date	
Preservative/container codes: A= plastic unfiltered, B= dark plastic, C= Syringe filtered, D=acidified unfiltered plastic, E= acidified filtered plastic, F=filter, G=glass unacidified, H=dark glass, I=ice, J=glass acidified, O=other, S=sterile plastic, V=VOA vial, W= plastic bag.										Data entry: _____	
Each preservative/container code represents one container ID. If more than one container is submitted for a code, enter the number of each beside the code.										Field review: _____	
Texas Institute for Applied Environmental Research Box T-0410, Stephenville, TX 76402, Tarleton State University 254-968-9570, 968-9560										Lab review: _____	
Q-110-1, rev. 3 mm 10/11/07											

CHAIN OF CUSTODY FOR KNOWN-SOURCE FECAL SAMPLES

Project Manager/Person(s) Requesting Sample					Sampler(s)		
Project Code	Sample No.	Test Group Code	Sample Date(s) (mm/dd/yy)	Sample Time(s) (hh:mm) CST for TIAER	Collection Site (GPS coord or town/city/county)	Animal Species	Other comments*

*If available, include other information such as:
1) Further description of animal – sex, age, species, etc.
2) For domesticated animals, further description of operation
 A. Beef v. dairy; if beef, cow-calf v. feedlot; if dairy, was sample collected from fresh manure, lagoon, etc.?
 B. For poultry, name of integrator, age of birds, etc.
3) For WWTP, type of system and treatment stage in plant where sample was collected.

Shipment of fecal samples from TIAER to SCSC			
Relinquished by:		Received by:	
Person	Date/Time	Person	Date/Time

Appendix C Example Corrective Action Report Form

Corrective Action Report

SOP-Q-105

CAR #: 08-003

Report Initiation Date _____ Report By: _____ Procedure or QC Typ _____

Deviation: _____

Analyte: _____

Affected Sample #s: _____

Sampling Station: _____

Project(s): _____

**Attached
 Documentation:**

- ☐ COC
- ☐ FDS
- ☐ FlowLink
- ☐ Flow8
- ☐ GM
- ☐ Log Book
- ☐ QC Sheet
- ☐ Memo
- ☐ Other

Details of the problem, nonconformance or out-of-control situation:

Possible Causes:

Corrective Actions Taken:

Corrective Actions Suggested:

CAR routed to: _____ Date: _____

Supervisor: ☐ Tier 1 (does not affect final data integrity) ☐ Tier 2 (data accepted but flag required) ☐ Tier 3 (possibly affects final data integrity)

Corrective actions taken for specific incident: _____

Corrective actions taken to prevent recurrences: _____

Corrective actions to be taken: _____

Responsible Party: _____ Proposed completion date: _____

Effect on data quality: _____

Responsible Supervisor: _____ Date: _____

Concurrence:

Program/Project Manager: _____ Date: _____
(Tier 3 CARs only)

Quality Assurance Officer: _____ Date: _____

Appendix D

SCSC SOPs for Sample Handling and Shipping and Analysis of BST and Known Source Samples

- D1: *Bacteroidales* PCR: Preprocessing of Water Samples
- D2: Isolation of *E. coli* from Water Samples: Preprocessing of Water Samples
- D3: Collection of Fecal Samples for Bacterial Source Tracking
- D4: Archival of *Escherichia coli* Isolates
- D5: ERIC-PCR of *Escherichia coli*
- D6: RiboPrinting of *Escherichia coli*
- D7: *Bacteroidales* PCR

D1: *Bacteroidales* PCR

Preprocessing of Water Samples

1. Within six hours of sample collection, water samples (100 ml total; 25 ml through 4 separate filters for each sample) are filtered through 0.2 µm pore size Supor-200 filters (VWR cat # 28147-979). It is recommended that disposable filter units (e.g., Nalgene Analytical Test Filter Funnels, VWR cat # 28198-861) be used in order to reduce the potential for cross-contamination between samples.
2. Discard filtrate and place each filter into a separate, pre-labeled Whirl-Pak bag (7 oz ; VWR cat # 66175-612) using ethanol-flamed forceps and aseptic technique. If 25 ml of water cannot be filtered, record the volume filtered on the Whirl-Pak bag and COC.
3. Add 500 µl of guanidine isothiocyanate (GITC) lysis buffer to each Whirl-Pak bag 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
4. Store samples at -80°C (or -20°C manual defrost freezer, not the standard auto-defrost)
5. Once per quarter (or more frequently), samples should be shipped overnight on dry-ice to SAML (address below). 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.
6. Notification of shipment should be sent to SAML (Emily Martin and Heidi Mjelde) via email, emartin@ag.tamu.edu and hmjelde@ag.tamu.edu, or phone, SAML Lab 979-845-5604, no later than the day of overnight shipping. Notification should include tracking number and direct TIAER contact person for confirmation upon receipt of samples.
7. Ship filters (and COCs) in insulated coolers with dry ice sufficient to keep samples frozen to:

Terry Gentry
Texas A&M University
Soil & Crop Sciences; Heep Center 539
370 Olsen Blvd
College Station, TX 77843
979-845-5604

D2: Isolation of *E. coli* from Water Samples

Preprocessing of Water Samples

1. Follow the EPA Method 1603 Modified mTEC procedure (EPA-821-R-06-011, Modified EPA Method 1603;
http://water.epa.gov/scitech/swguidance/methods/bioindicators/upload/2008_11_25_methods_method_biological_1603.pdf).
2. After 22 +/- 2 hour incubation, red or magenta colonies are considered 'typical' *E. coli*.
3. 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.
4. After counting, the plates should be immediately stored at 4°C until shipment in order to prevent growth of non-*E. coli* coliforms on the plates.
5. In preparation for shipping, 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.
6. The plates should be shipped as soon as possible (preferably the day after filtration, but no later than three days following filtration) to SAML (address below) at 4°C. '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.
7. If sampling occurs over two days, the first day's plates should be counted 24 hours post filtration, sealed and placed 'media-side up' (i.e. upside down), so condensation does not fall onto the filter, and stored at 4°C until a complete sample set can be shipped together on the next day.
8. Notification of shipment should be sent to SAML (Emily Martin and Heidi Mjelde) via email, emartin@ag.tamu.edu and hmjelde@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 TIAER contact person for confirmation upon receipt of samples.
9. Ship plates (and COCs) in insulated coolers with sufficient ice packs to maintain ~4°C to:

Terry Gentry
Texas A&M University
Soil & Crop Sciences; Heep Center 539
370 Olsen Blvd
College Station, TX 77843
979-845-5604

D3: Collection of Fecal Samples for Bacterial Source Tracking

1. Only fresh fecal samples of known origin should be collected. Specifically, fecal samples should be obtained in one of four ways:
 - a. Collected from intestines of animals legally harvested.
 - b. Collected from animals visually observed defecating by technician.
 - c. Collected from the intestines of animals recently killed by cars (within 24 hours).
 - d. Human (wastewater) samples collected from septic tanks or from influent (pre-secondary treatment) at wastewater treatment plants. Alternatively, fecal samples can be collected from individual people.
2. Samples should be carefully collected to avoid contamination. Samples on the ground should be collected with a sterile spatula, or similar device, while avoiding collection of material in contact with soil or other possible sources of contamination. Intestinal samples should be collected from animals by using sterile loops inserted anally or by cutting into the intestine using a sterile scalpel. Wastewater samples can initially be collected with sterile bottles, or other suitable device and then transferred to the fecal tubes described below.
3. Each fecal sample should be placed in a new, sterile fecal tube (Sarstedt, cat# 80.734.311). Tubes should be filled approximately $\frac{3}{4}$ full (can provide less material for smaller animals).
4. Samples should be refrigerated ($\sim 4^{\circ}\text{C}$) or kept on ice following collection.
5. At the time of sampling, record detailed information regarding the sample including:
 - a. Sampling date
 - b. Animal species
 - c. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
 - d. Sample collector's name/initials
 - e. Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle
6. Notify SAML (Emily Martin and Heidi Mjelde) via email (emartin@ag.tamu.edu and hmjelde@ag.tamu.edu) or phone (SAML Lab 979-845-5604) as soon as possible (prior to or immediately following sample collection) with an estimated number of samples that will be shipped and the expected date of shipment. This will allow SAML to make appropriate preparations to process the samples immediately upon arrival.
7. Samples should be shipped (at 4°C) as soon as possible (within 5 days) to SAML (address below). 'Blue-ice' or freezer blocks should be used to keep the samples cool, but not frozen during transport. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.
8. Notification of shipment should be sent to SAML (Emily Martin and Heidi Mjelde) via email (emartin@ag.tamu.edu and hmjelde@ag.tamu.edu) or phone (SAML Lab 979-845-5604) no later than the day of overnight shipping. Notification should include tracking number and direct TIAER contact person for confirmation upon receipt of samples.

9. Ship samples (and COCs) in insulated coolers (marked on outside to indicate that contents are perishable) with sufficient ice packs to maintain $\sim 4^{\circ}\text{C}$ to:

Terry Gentry
Texas A&M University
Soil & Crop Sciences; Heep Center 539
370 Olsen Blvd
College Station, TX 77843
979-845-5604

D4: 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.

D5: 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 Cyclor 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

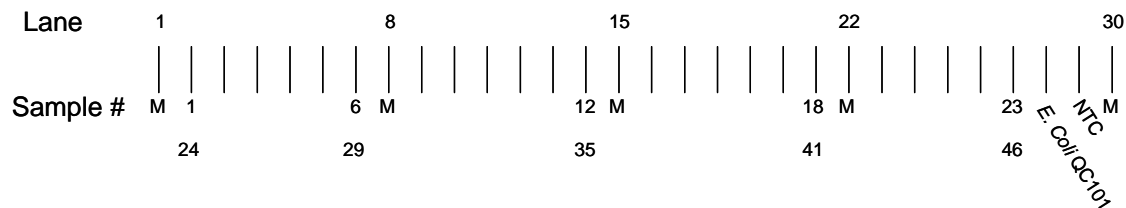
62.5 µL ethidium bromide (Sigma, 10 mg/mL)

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.

D6: 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

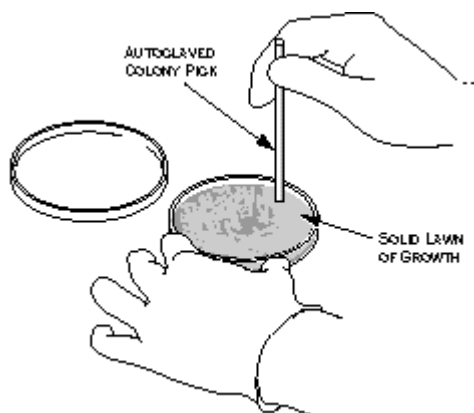
1. Place a sterile 0.65 mL microfuge tube in each of the eight holes in the lower row of the sample preparation rack.
2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.

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. For Gram negative samples (e.g. *E. coli*), perform 1 pick placed into 200 μ L of sample buffer.

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*.

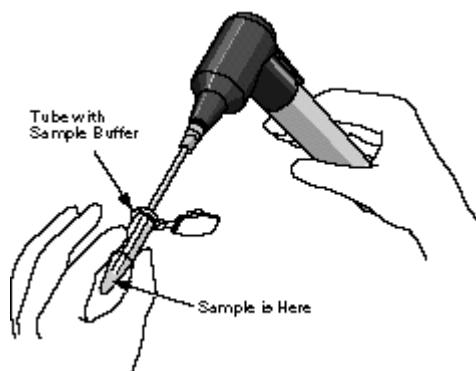
5. Mix the Samples

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 μ L pipetter, pipette 30 μ L 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.

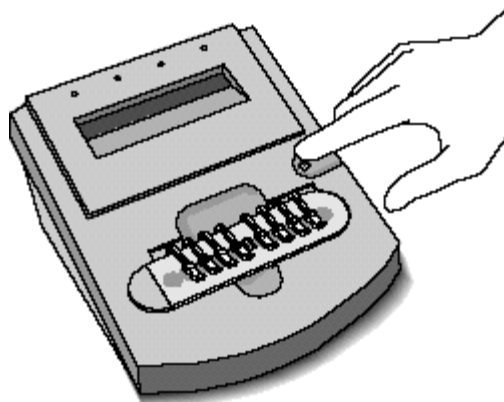
CAUTION! Transfer the sample carrier to the Heat Treatment Station within 2 hours. If you wait longer than 2 hours, you will have to discard the sample carrier and begin again for this batch.

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

6. 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:

- [EcoRI batches \(VCA\)](#)
- [PstI batches \(VCB\)](#)
- [PvuII batches \(VCC\)](#)

You can also create special batches:

- Restriction Enzyme Flexibility batches
- **Substitute Enzyme batches (including *Hind 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.

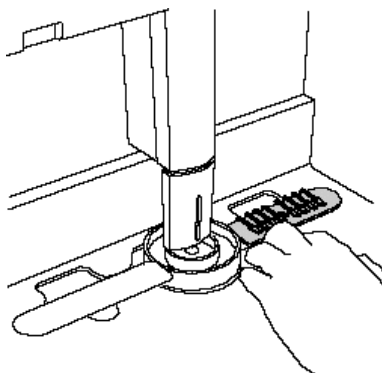
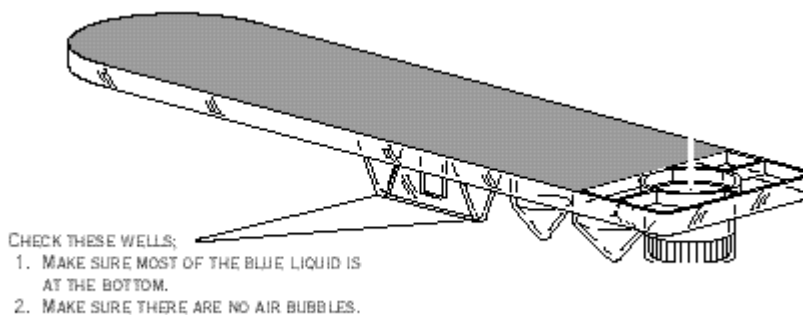
3. Load the DNA Prep Carrier

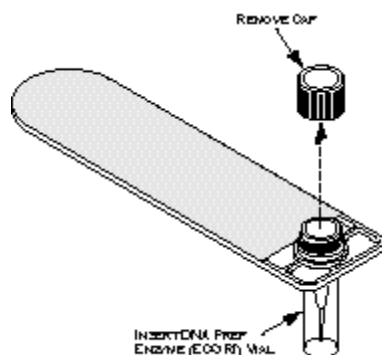
1. Remove the DNA Prep carrier from the refrigerator.
2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has 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 (*Hind* III or *Eco*R I) from the freezer. ***Hind* III (NEB Cat. #R0104M) is prepared in a Sarstedt 500- μ L microfuge tube (Cat. #72730-005) as a 50 U/ μ L working stock as follows.**

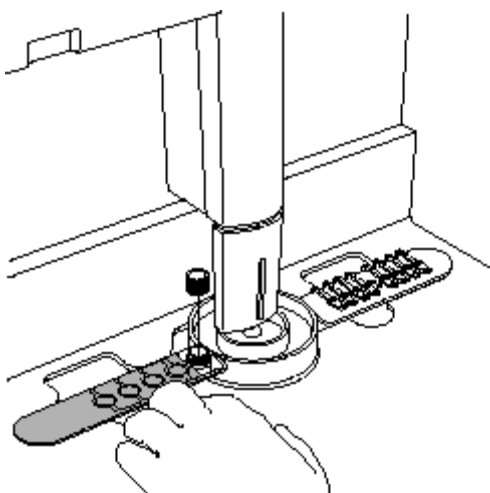
50 U/ μ L: 26.5 μ L *Hind* III and 26.5 μ L of NEB 10X Buffer 2

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





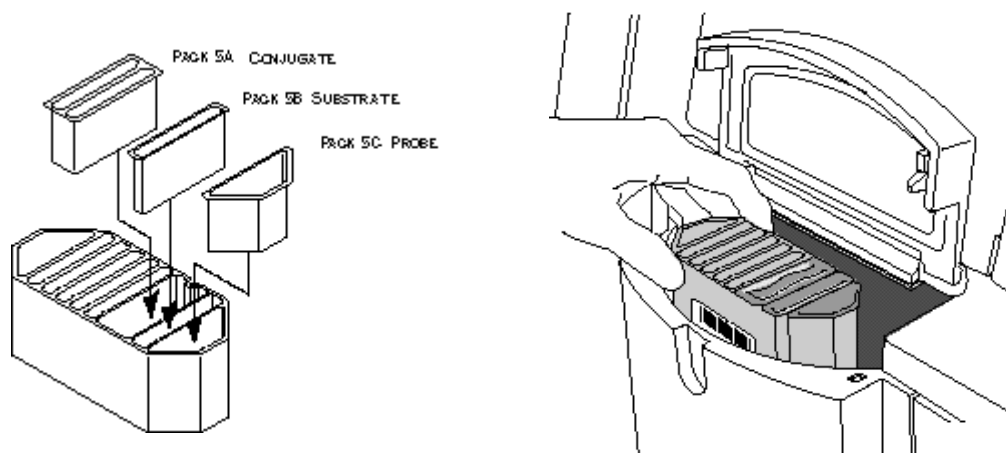
5. Remove the cap from the Enzyme vial.
6. Insert the vial into the carrier.
7. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.
8. Push the DNA Prep carrier down firmly until it snaps into place.



4. Load the MP Base and Carousel

1. Unpack the disposables.

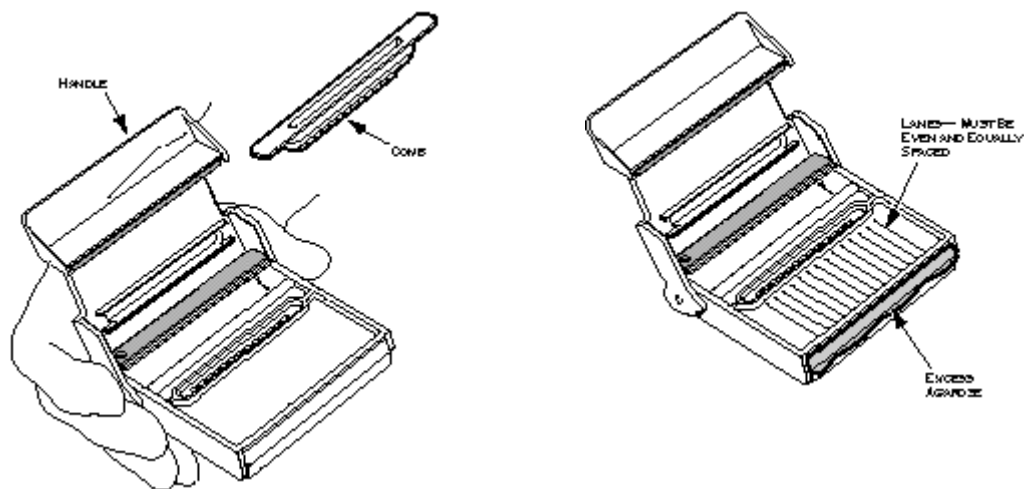
2. Remove the MP base (Pack 5) from the incubator and the Conjugate (Pack 5A), Substrate (Pack 5B), and Probe (Pack 5C) from the refrigerator.
3. Remove each insert from its pouch. Tap the powdered reagent packs gently to bring all powder to the bottom of the packs. Place reagent packs in the MP base 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.

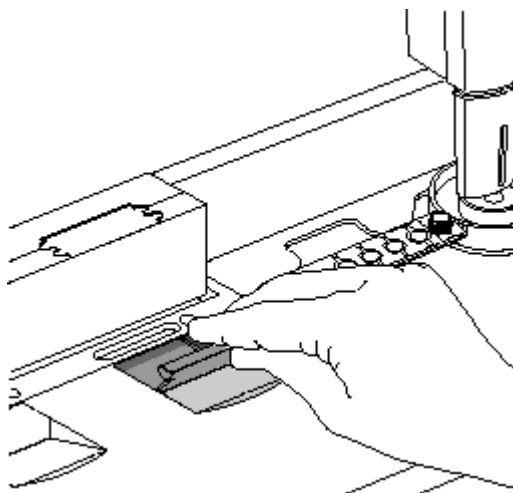
5. Load the Gel Cassette

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.

1. 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.

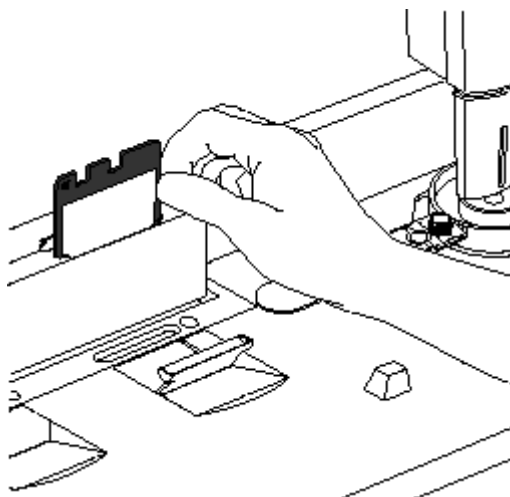


2. Press the cassette forward firmly until it snaps into place.

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

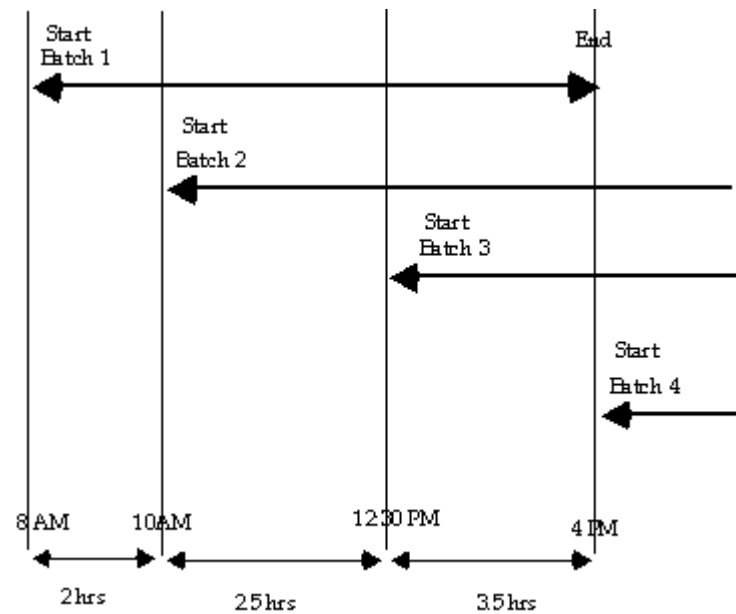
The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.

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.

D7: *Bacteroidales* PCR

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
MgCl ₂ (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:

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 Loading Buffer (see recipe below)

150 µL 10X PCR buffer

850 µL molecular grade water

Store in cold room

6X 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

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.

Appendix E

Data Review and Summary Checklist

DATA SUMMARY CHECKLIST

A completed checklist must accompany all data sets submitted to the TSSWCB by TIAER.

Data Format and Structure Y,N, or N/A

- A. Are there any duplicate Tag_Ids in the Events file?
- B. Are all StationIds associated with assigned station location numbers?
- C. Are all dates in the correct format, MM/DD/YYYY?
- D. Are all times based on the 24 hour clock format, HH:MM?
- E. Is the Comment field filled in where appropriate (e.g. unusual occurrence, sampling problems)?
- F. Are Source1, Source2 and Program codes used correctly?
- G. Do the Enddates in the Results file match those in the Events file for each Tag_Id?
- H. Are all measurements represented by a valid parameter code with the correct units? I. Are there any duplicate parameter codes for the same Tag_Id?
- J. Are there any invalid symbols in the Greater Than/Less Than (GT/LT) field?
- K. Are there any tag numbers in the Result file that are not in the Event file?
- L. Have verified outliers been identified with a "1" in the Remark field?

Data Quality Review

- A. Are all the "less-than" values reported at or below the specified reporting limit?
- B. Have checks on correctness of analysis or data reasonableness performed?
- C. Have at least 10% of the data in the data set been reviewed against the field and laboratory data sheets?
- D. Are all parameter codes in the data set listed in the QAPP?
- E. Are all StationIds in the data set listed in the QAPP?

Documentation Review

- A. Are blank results acceptable as specified in the QAPP?
- B. Was documentation of any unusual occurrences that may affect water quality included in the Event table's Comments field?
- C. 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.
- D. 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.

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

Submitted by: Date Submitted to TSSWCB:

TAG Series:

Date Range:

Data Source:

Comments (attach file if necessary):

Appendix F
TIAER Flow Measurement SOPs
(provided as separate pdf file)

Appendix G

RUAA Contact Information, Field Data, Interview and Summary Forms

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.

Notify TCEQ Water Quality Standards Group (required):

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: _____

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Date Notified: _____

Name: _____
Entity: _____
Date Notified: _____

Field Data Sheets – Basic RUAA Survey

(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: _____ °C Water Temp: _____ °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 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. 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).
 - ☐ 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 ☐ Yearly
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.

- | | |
|---|---|
| <input type="checkbox"/> Drinking or water in mouth | <input type="checkbox"/> Playing on shoreline |
| <input type="checkbox"/> Bathing | <input type="checkbox"/> Picnicking |
| <input type="checkbox"/> Walking | <input type="checkbox"/> Motorcycle/ATV |
| <input type="checkbox"/> Jogging/running | <input type="checkbox"/> Hunting/Trapping |
| <input type="checkbox"/> Bicycling | <input type="checkbox"/> Wildlife watching |
| <input type="checkbox"/> Standing | <input type="checkbox"/> None |
| <input type="checkbox"/> Sitting | <input type="checkbox"/> Other: _____ |
| <input type="checkbox"/> 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).

- | | | | | |
|---------------------------------------|---|---|--------------------------------------|--|
| <input type="checkbox"/> Culverts | <input type="checkbox"/> Fences | <input type="checkbox"/> Log jams | <input type="checkbox"/> Rip rap | <input type="checkbox"/> Water control structure |
| <input type="checkbox"/> Barbed wire | <input type="checkbox"/> Dams | <input type="checkbox"/> Thick vegetation | <input type="checkbox"/> Low bridges | <input type="checkbox"/> None |
| <input type="checkbox"/> Utility pipe | <input type="checkbox"/> Other (specify): _____ | | | |

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

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

Comments: _____

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

- | | | |
|---|--|---|
| <input type="checkbox"/> Private Property | <input type="checkbox"/> Fence | <input type="checkbox"/> No trespass sign |
| <input type="checkbox"/> Barge/ship traffic | <input type="checkbox"/> Wildlife | <input type="checkbox"/> Industrial |
| <input type="checkbox"/> Steep slopes | <input type="checkbox"/> None of the Above | <input type="checkbox"/> No public access |
| <input type="checkbox"/> Other: _____ | <input type="checkbox"/> No roads | |

Comments: _____

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

- | | | | |
|--|---|--|--|
| <input type="checkbox"/> Roads | <input type="checkbox"/> RV/ATV Tracks | <input type="checkbox"/> NPDES Discharge | <input type="checkbox"/> Organized event |
| <input type="checkbox"/> Rope swings | <input type="checkbox"/> Camping Sites | <input type="checkbox"/> Gates on corridor | <input type="checkbox"/> No Human Presence |
| <input type="checkbox"/> Dock/platform | <input type="checkbox"/> Fire pit/ring | <input type="checkbox"/> Children's toys | |
| <input type="checkbox"/> Foot paths/prints | <input type="checkbox"/> Fishing Tackle | <input type="checkbox"/> Remnant's of Kid's play | |
| <input type="checkbox"/> 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).

[illegible]

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, how many years? _____
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 7Q2 equal 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 water body.

Name of waterbody: _____

Segment # or Nearest Downstream Segment #: _____

Classified Segment?: _____

County: _____

1. Observations on Use

a. Do primary contact recreation activities occur on the water body?

☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

b. Do secondary contact recreation 1 activities occur on the water body?

☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

c. Do secondary contact recreation 2 activities occur on the water body?

☐ frequently ☐ seldom ☐ not observed or reported ☐ unknown

d. Do noncontact recreation activities occur on the water body?

☐ 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

