Total Maximum Daily Load Grant Program

Bacterial Source Tracking to Support the Development and Implementation of Watershed Protection Plans for the Lampasas and Leon Rivers

TSSWCB Project # 10-51
Revision 1

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

prepared by

Texas AgriLife Research - Texas Water Resources Institute
Texas AgriLife Research and Extension Center at El Paso
Texas AgriLife Research, Blackland Research and Extension Center at Temple

Effective Period: August 1, 2010 to July 31, 2012
with annual updates required

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

Lucas Gregory
Quality Assurance Officer
Texas AgriLife Research
Texas Water Resources Institute
2118 TAMU
College Station, TX 77843-2118
lfgregory@ag.tamu.edu
979.845.7869

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Section A1: Approval Sheet

Bacterial Source Tracking to Support the Development and Implementation of Watershed Protection Plans for the Lampasas and Leon Rivers

Texas State Soil and Water Conservation Board (TSSWCB)

Name: Pamela Casebolt
Title: TSSWCB Project Manager (PM)

Signature: ______________________________ Date:___________________

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

Signature: ______________________________ Date:___________________

Texas AgriLife Research - Texas Water Resources Institute (TWRI)

Name: B. L. Harris
Title: TWRI, Acting Director; Project Coordinator

Signature: ______________________________ Date:___________________

Name: Lucas Gregory
Title: TWRI QAO

Signature: ______________________________ Date:___________________

Texas AgriLife Research and Extension Center at El Paso (AgriLife-EP)

Name: George Di Giovanni, Ph.D.
Title: Professor and Faculty Fellow; Project Co-Leader

Signature: ______________________________ Date:___________________

Texas AgriLife Research - Blackland Research and Extension Center at Temple (AgriLife-TP)

Name: June Wolfe, Ph.D.
Title: Assistant Research Scientist; Project Co-Leader

Signature: ______________________________ Date:___________________

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List of Acronyms and Abbreviations

AgriLife-EP	Texas AgriLife Research and Extension Center at El Paso
AgriLife-TP	Texas AgriLife Research - Blackland Research and Extension Center at Temple
AWRL	Ambient Water Reporting Limit
BHI	Brain-Heart Infusion
BMPs	best management practices
BST	bacterial source tracking
CAR	corrective action report
CR	county road
CFU	colony forming units of bacteria
COC	chain of custody
CRP	Texas Clean Rivers Program
CWA	Clean Water Act
DO	dissolved oxygen
DNA	deoxyribonucleic acid
DQO	data quality objectives
E. coli	Escherichia coli
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction
ERIC-RP	ERIC-PCR and RiboPrinting combination method
FM	Farm to Market Road
GPS	global positioning system
MP	membrane and probe
mTEC	membrane Thermotolerant *E. coli*
MUG	4-methylumbelliferyl-β-D-glucuronide
NA-MUG	Nutrient Agar 4-methylumbelliferyl-β-D-glucuronide
NELAC	National Environmental Laboratory Accreditation Conference
NIST	National Institute of Standards and Technology
NPS	nonpoint source
PCR	polymerase chain reaction
PM	project manager
QA	quality assurance
QAO	quality assurance officer
QAPP	quality assurance project plan
QC	quality control
qPCR	quantitative PCR
QPR	quarterly progress report
RP	RiboPrinting
RPD	relative percent deviation
SH	state highway
SM	Standard Methods for the Examination of Water and Wastewater
SOP	Standard Operating Procedure
SWCD	Soil and Water Conservation District
TBE	Tris/Borate/EDTA Buffer
TCEQ	Texas Commission on Environmental Quality
TMDL	total maximum daily load
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas Water Resources Institute
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV	ultraviolet light
WPP	Watershed Protection Plan

Section A3: Distribution List

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

Texas State Soil and Water Conservation Board

PO Box 658
Temple, TX 76503

Name: Pamela Casebolt
Title: TSSWCB PM

Name: Donna Long
Title: TSSWCB QAO

Texas AgriLife Research - Texas Water Resources Institute

1500 Research Parkway, Suite 240
2118 TAMU
College Station, TX 77843-2118

Name: B. L. Harris
Title: TWRI Acting Director; Project Coordinator

Name: Lucas Gregory
Title: TWRI QAO

Texas AgriLife Research - Blackland Research and Extension Center at Temple

720 E Blackland Rd
Temple, TX 76502

Name: June Wolfe, Ph.D.
Title: Assistant Research Scientist, Project Co-Leader

Texas AgriLife Research and Extension Center at El Paso

1380 A&M Circle
El Paso, TX 79927-5020

Name: George Di Giovanni, Ph.D.
Title: Professor and Faculty Fellow, Project Co-Leader

Section A4: Project/Task Organization

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

TSSWCB – Provides state oversight and management of all project activities and ensures coordination of activities with related projects.

Pamela Casebolt, TSSWCB PM
Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Tracks and reviews deliverables to ensure that tasks in the workplan are completed as specified.

Donna Long, TSSWCB QAO
Reviews and approves the QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB and project participants. Responsible for verifying that the QAPP is followed by project participants. Determines that the project meets the requirements for planning, quality assurance/quality control (QA/QC), and reporting. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures.

TWRI – Project coordination and management of all project activities and ensure coordination of activities with related projects.

B. L. Harris, TWRI Acting Director; Project Coordinator
Responsible for ensuring that tasks and other requirements in the contract are executed on time and as defined by the grant workplan; assessing the quality of work by participants; submitting accurate and timely deliverables and costs to the TSSWCB; and coordinating attendance at conference calls, meetings, and related project activities.

Lucas Gregory, TWRI QAO
Responsible for determining that the QAPP meets the requirements for planning, QA/QC, and reporting activities conducted by TWRI. Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract workplan and in the QAPP. Responsible for the facilitation of audits and the implementation, documentation, verification, and reporting of corrective actions.

AgriLife-TP – Responsible for water sample collection and preparation, streamflow monitoring, known source fecal sample collection.

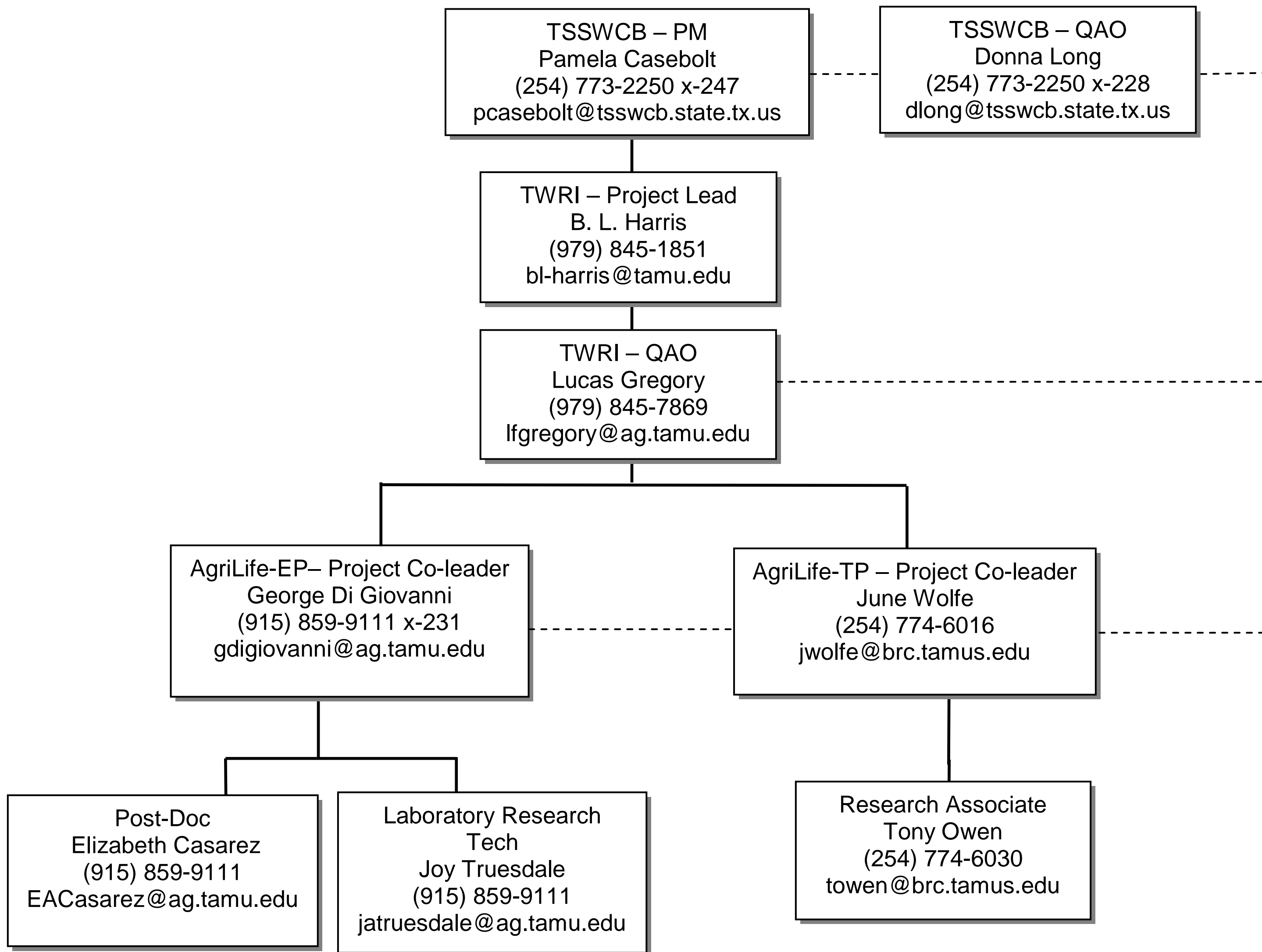
Dr. June Wolfe, Assistant Research Scientist, Project Co-Leader

Responsible for coordinating and supervising field sampling activities. Responsible for ensuring that field personnel have adequate training on equipment and methods specific to the analysis or task performed and/or supervised. Responsible for verifying that the data produced are of known and acceptable quality. Responsible for ensuring adequate training and supervision of all activities involved in generating analytical data for this project. Responsible for general facilitation of audits and reporting of corrective actions. Responsible for submitting accurate and timely data analyses and other materials for QPRs and final reports to TWRI.

AgriLife-EP – Conduct BST and incorporate known source fecal *E. coli* isolates into the Texas *E. coli* BST Library, compile BST results into technical reports delineated by watershed.

Dr. George D. Di Giovanni, Professor and Faculty Fellow, Project Co-Leader
Responsible for conducting *E. coli* ERIC-PCR and RiboPrinting BST analyses to determine the human and animal influence of bacterial loading to the creek and the need to augment the Texas *E. coli* BST Library with samples from the Lampasas and Leon River watersheds. Responsible for technical oversight of activities involved in generating analytical data by the AgriLife-EP laboratory. Responsible for general facilitation of audits and reporting of corrective actions. Responsible for submitting accurate and timely data analyses and other materials for QPRs and final reports to TWRI.

Figure A.4-1. Project Organization Chart



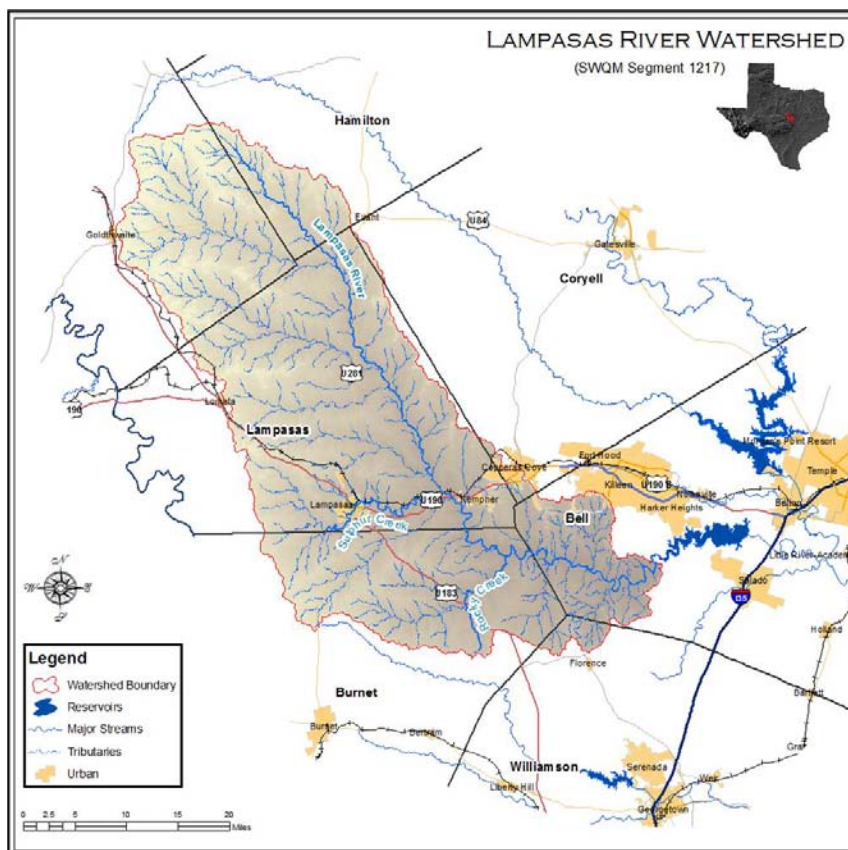
Section A5: Problem Definition/Background

Lampasas River

The Lampasas River (segment 1217 in the Brazos River Basin), rises in western Hamilton county 16 miles west of Hamilton and flows southeast for 75 miles, passing through Lampasas, Burnet, and Bell counties. In Bell County the river turns northeast and is dammed five miles southwest of Belton to form Stillhouse Hollow Lake (segment 1216). Below Stillhouse Hollow Lake, the Lampasas River flows to its confluence with Salado Creek and the Leon River to form the Little River.

The Lampasas River is commonly characterized by relatively low water levels and is situated within a predominantly rural and agricultural landscape. Land use within the watershed is dominated by rangeland and grasslands. Major agricultural interests include the production of beef cattle on rangeland, hay, wheat, oats, sorghum, corn, cotton, peanuts, and pecans.

Figure A.5-1. Lampasas River Watershed Map



Recreational uses of the waterbody are considered to be impaired. Bacteria exceed established criteria. These organisms are generally not harmful to human health, but may indicate the

presence of pathogens that can cause disease or gastrointestinal illnesses. The Lampasas River above Stillhouse Hollow Lake is listed on the *2008 Texas 303(d) List* for elevated bacteria levels.

The data used to assess bacterial concentrations in the Lampasas River is the result of sampling conducted in 1998-1999 through the Clean Rivers Program. Fecal coliform samples were taken at 5 designated sampling sites along the Lampasas River, which resulted in two of the five sampling sites indicating a use concern or non-support of contact recreation. The draft 2010 Integrated Report has delisted the Lampasas River due to the data no longer meeting current data criteria. *E. coli* samples continue to be collected in the watershed through the Clean Rivers Program. TCEQ has also initiated a 24 month monitoring project at site 17750 in the upper portion of the watershed; the location that was listed as not meeting surface water quality standards for bacteria.

Although routine sampling indicates the presence of elevated bacteria levels in the Lampasas River, the origin of this source is unclear. In order to shed light on the sources contributing to the Lampasas River bacteria impairment, library-dependent BST is needed. This approach will utilize proven scientific methods that will discriminate between the various sources of bacteria. When the sources have been identified, appropriate management measures can be implemented for the respective sources.

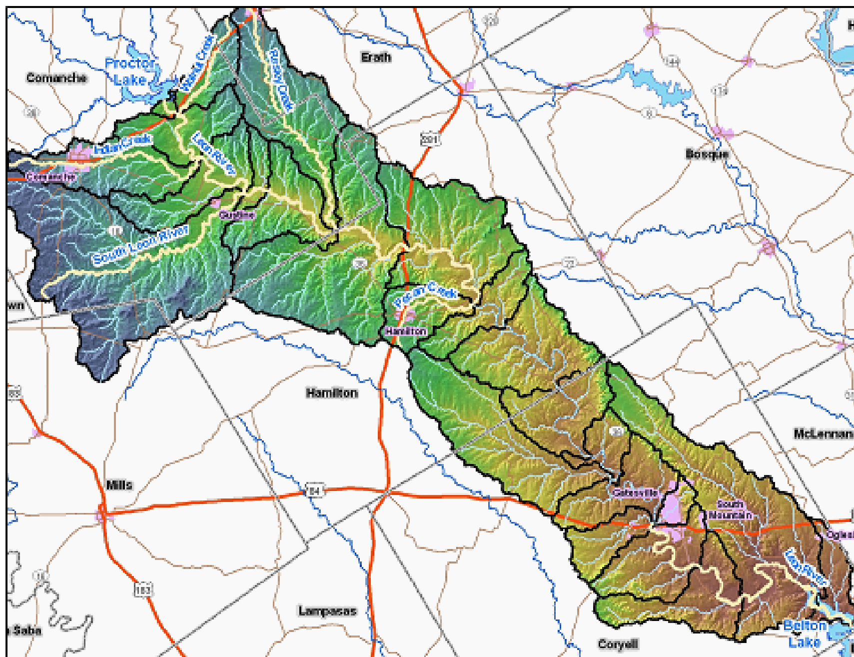
A Watershed Protection Plan (WPP) for the Lampasas River is being developed through TSSWCB project, 07-11— *Lampasas River Watershed Assessment and Protection Project*. AgriLife-TP also serves as the TSSWCB-contracted watershed coordinator for this WPP process. The development of the Lampasas River WPP is independent of this BST project; however, conclusions from this BST project will be integrated into the WPP through adaptive management.

Leon River

The Leon River watershed below Proctor Lake and above Belton Lake encompasses approximately 1,362 square miles in Bell, Hamilton, Coryell, Comanche, Erath and Mills counties. In 1996, the entire Leon River was placed on the *Texas 303(d) List* for bacteria levels that “sometimes exceed water quality standards”. In 1996, the entire Leon River below Lake Proctor (Segment 1221) was placed on the *303(d) List* for bacteria levels “Not Supporting Contact Recreation Use”. The 2008 *303(d) List* identified all but two of the segment’s assessment units as impaired or having a concern for near non-attainment resulting from elevated *E. coli levels.* Additionally, five tributaries of the Leon River are impaired for bacteria (1221A – Resley Creek, 1221B – South Leon River, 1221C – Pecan Creek, 1221D – Indian Creek, 1221F – Walnut Creek).

The Leon River watershed is a predominantly rural, agricultural watershed that primarily houses rangeland and row crop agricultural practices. Forests also cover a sizable amount of the watershed. A significant amount of dairy production also exists in the northern portion of the watershed.

Figure A.5-2. Leon River Watershed Map



A draft TMDL developed by TCEQ, but not adopted, identified sources of bacterial pollution in the Leon River watershed as wastewater treatment facility discharges, stormwater runoff, failing septic systems, wildlife and feral animals, as well as fecal deposition from livestock and pets. Water quality data support the assumption that these sources of bacterial contamination are present and contributing to the bacterial loading of the river. Through the TMDL development process that utilized water quality monitoring, computer based modeling, watershed assessments and limited, library-dependent BST; TCEQ determined that a 21% load reduction in bacteria levels would be needed to restore water quality in the Leon River.

Leon watershed stakeholders sought to initiate the development of a Leon River WPP during the course of the TMDL development process, which was accommodated via TSSWCB project 06-12, *Leon River Watershed Protection Plan Project*. The Brazos River Authority serves as the TSSWCB-contracted watershed coordinator for this project. Development of the Leon River WPP will proceed independent of this BST project; however, conclusions from this BST project will be integrated into the WPP through adaptive management.

In an effort to identify what potential sources are contributing to the bacterial loading of the Leon River, library-dependent BST will be re-employed. Advances in BST technologies and techniques since the original BST assessment was conducted for the TMDL and inclusion of additional sampling stations will produce higher certainty results which will be used to determine the most appropriate management measures needed.

Section A6: Project/Task Description

General Project Description

This project consists of two major components: 1) the collection of water samples in conjunction with streamflow measurements, and 2) the utilization of BST on those collected water samples. Building on work being conducted in the Lampasas River watershed (TSSWCB project 07-11) and the Leon River watershed (TSSWCB project 06-12), this project will apply portions of the TCEQ- and TSSWCB-approved Three-Tier Approach for Developing Bacteria TMDLs, as recommended by the joint Bacteria TMDL Task Force. This project will provide critical bacteria loading information as well as source identification information through the utilization of library-dependent BST which will be used to support the development and implementation of WPPs for the Lampasas and Leon Rivers watersheds.

AgriLife-TP will conduct all environmental water sample collection and monitoring. AgriLife-TP will obtain and maintain NELAC® accreditation for laboratory methods used in this project. *E. coli* levels in collected water samples will be enumerated utilizing USEPA method 1603; flow rates and field parameters will be collected in conjunction with water samples. Samples will be collected at 30 sites - 15 in the Lampasas River watershed and 15 in the Leon River watershed - monthly for a period of 12 months. Tentative sampling locations in each watershed are listed in Tables 1 and 2. After *E. coli* enumeration, AgriLife-TP will ship the enumerated plates to AgriLife-EP for BST. AgriLife-TP will also collect 50 known source fecal samples per watershed (total of 100) from sources identified by AgriLife-EP. Known source fecal samples will be collected and shipped to AgriLife-EP using methodology described in Appendix E where they will be processed using USEPA method 1603 to culture, enumerate, and isolate *E. coli* colonies.

Table A6-1. Leon River Watershed Sampling Locations

RD= Road; CR= County Road; FM= Farm to Market Road; SH= State Highway

TCEQ Station_ID	Location	Latitude	Longitude
11934	LEON RIVER AT US 67/US 377	31.95778	-98.4593
17379	WALNUT CREEK AT FM 1476	31.97312	-98.4367
11933	LEON RIVER AT COMANCHE CR 382	31.82971	-98.2575
11818	INDIAN CREEK AT CR 304	31.88658	-98.4381
11808	RESLEY CREEK AT COMANCHE CR394	31.81303	-98.2240
11930	LEON RIVER AT CR 431	31.60882	-97.8968
11817	SOUTH LEON RIVER AT SH 36	31.84813	-98.3708
11929	LEON RIVER AT CORYELL CR 183	31.52514	-97.8601
17547	PECAN CREEK AT SH 22	31.71031	-98.0563
18405	PLUM CREEK AT CORYELL CR 106	31.5126	-97.9001
11932	LEON RIVER AT US 281	31.78746	-98.1211
11804	CORYELL CREEK AT FM 107	31.39278	-97.5994
17501	LEON RIVER AT FAUNT LEROY PARK (GATESVILLE)*	31.46250	-97.7492
11928	LEON RIVER AT US 84**	31.43503	-97.7595
11926	LEON RIVER AT SH 36	31.38369	-97.7017
11925	LEON RIVER AT FM 1829	31.33584	-97.6425

* Flow determined from upstream USGS gauge (TCEQ 11928) when stream cannot be safely waded.
**Alternate flow data station for TCEQ 17501 during periods of high flow. No other data used from this station.

Table A6-2. Lampasas River Watershed Sampling Locations

RD= Road; CR= County Road; FM= Farm to Market Road; SH= State Highway

TCEQ Station_ID	Location	Latitude	Longitude
15762	LAMPASAS RIVER AT US 84	31.48027	-98.2735
15770	LAMPASAS RIVER AT LAMPASAS CR 2925	31.37584	-98.1798
TBD	BENNETT CREEK AT LAMPASAS CR 2901	31.40775	-98.2395
16404	LAMPASAS RIVER AT FM 2313	31.11900	-98.0565
11872	SULPHUR CREEK AT NARUNA RD	31.05040	-98.1852
15781	SULPHUR CREEK AT LAMPASAS CR 3010	31.07091	-98.1353
TBD	MESQUITE CREEK AT LAMPASAS CR 4390	31.05357	-98.0487
11897	LAMPASAS RIVER AT US 190	31.08167	-98.0164
11724	ROCKY CREEK AT FM 963	30.98541	-97.9266
18759	REESE CREEK NR FM 2670 BR985	30.97930	-97.7847
11896	LAMPASAS RIVER AT SH 195	30.97248	-97.7786
TBD	CLEAR CREEK AT OAKALLA RD	31.00634	-97.8887
TBD	SCHOOL CREEK AT US 281	31.21433	-98.1731
15763	SIMMS CREEK AT US 281	31.26815	-98.1746
15250	SULPHUR CREEK AT LAMPASAS CR 3050	31.08544	-98.0507

Water samples collected by AgriLife-TP will be enumerated for *E. coli* and shipped to AgriLife-EP for BST analysis. Library-dependent BST will be conducted on samples from both the Lampasas and Leon Rivers watersheds by analyzing *E. coli* isolates utilizing the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) and RiboPrinting (RP) combination method (ERIC-RP). Known source fecal samples from the study area collected by AgriLife-TP will also be processed for ERIC-RP analysis by AgriLife-EP for inclusion in the Texas *E. coli* BST Library.

TWRI, AgriLife-EP, and AgriLife-TP will develop Technical Reports (one per watershed) summarizing water quality data and BST analysis. AgriLife-EP and AgriLife-TP will present results to the existing WPP stakeholder groups. AgriLife-EP will address and reconcile discrepancies between BST results from this project and modeling outcomes from TSSWCB projects 06-12 and 07-11.

TWRI will transfer monitoring data (bacteria, field, and flow parameters), appropriately formatted, to TSSWCB for inclusion in the TCEQ Surface Water Quality Monitoring Information System (SWQMIS).

TWRI, AgriLife-TP, and AgriLife-EP will participate in Lampasas River and Leon River WPP meetings, Clean Rivers Program Steering Committee meetings, and other meetings as appropriate to coordinate monitoring efforts, and provide summarized activities and achievements made through this project.

TWRI will develop a Quality Assurance Project Plan (QAPP) for work conducted under Tasks 3 and 4 to ensure data of known and acceptable quality are generated and used in this project. The QAPP will be consistent with *EPA Requirements for Quality Assurance Project Plans (QA/R-5)*, the *TSSWCB Environmental Data Quality Management Plan*, TSSWCB-approved SOPs for BST methods (ERIC-PCR and RP), and TCEQ's *Surface Water Quality Monitoring Procedures (Oct 2008).*

Figure A.6-1. Flow Diagram of Experimental Approach for BST

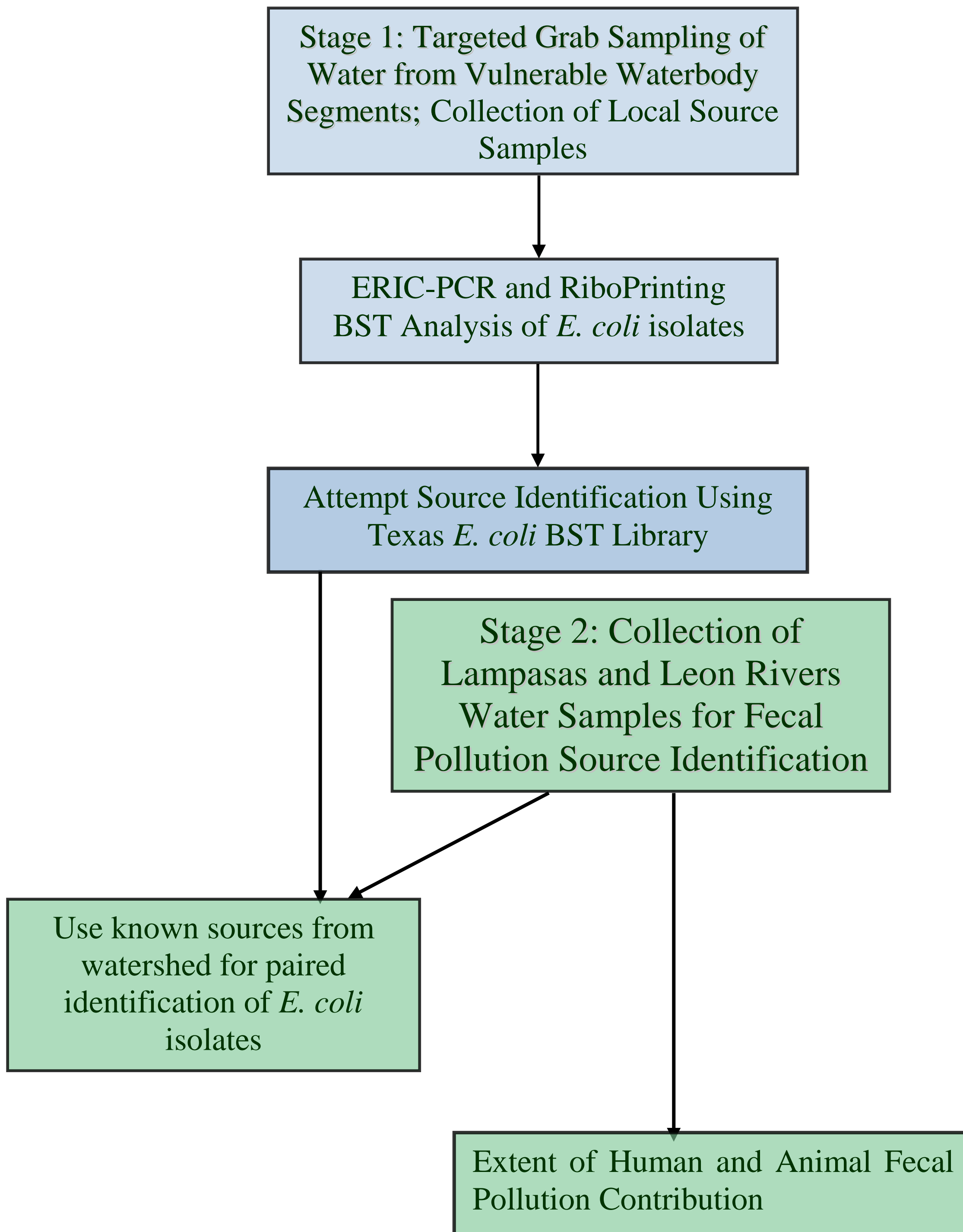


Table A.6-3. Project Plan Milestones

TASK	PROJECT MILESTONES	AGENCY	START	END
3.1a	Conduct sampling site reconnaissance at prospective sites to determine suitability for sample collection	AgriLife-TP, TSSWCB, AgriLife-EP	Aug 10	Oct 10
3.1b	Submit Station Location Requests (SLOCs) to TCEQ as needed to obtain TCEQ station numbers for new monitoring sites	TWRI, AgriLife-TP	Aug 10	Oct 10
3.2	Perform routine monitoring at 15 sites in the Leon River Watershed and 15 sites in the Lampasas River watershed monthly, collecting field, flow, and bacteria parameter groups for 12 months	AgriLife-TP	Nov 10	Oct 11
3.3	Enumerate and record *E. coli* colonies in water samples	AgriLife-TP	Nov 10	Oct 11
3.4	Coordinate shipment of samples to AgriLife-EP to ensure that samples are received in El Paso within 3 days of analysis	AgriLife-TP	Nov 10	Oct 11
3.5a	Collect approx. 50 known source fecal samples from each watershed (100 total) for shipment to AgriLife-EP for *E. coli* isolation analysis	AgriLife-TP	Oct 10	Jan 11
3.5b	Coordinate shipment of samples to AgriLife-EP to ensure that samples are received in El Paso within 3 days of analysis	AgriLife-TP	Nov 10	Oct 11
3.6a	Develop technical reports for data collection and flow monitoring in each watershed	AgriLife-TP, AgriLife-EP, TWRI	Nov 11	Jul 12
3.6b	Participate in appropriate Leon and Lampasas Rivers Stakeholder meetings to present results from data collection activities	AgriLife-TP	Nov 11	Jul 12
3.7a	Transfer quarterly monitoring data to TSSWCB for inclusion into TCEQ SWQMIS	TWRI, AgriLife-TP	Nov 10	Jan 12
3.7b	Provide necessary information on monitoring regime to BRA for inclusion in the Coordinated Monitoring Schedule	TWRI	Nov 10	Jan 12
4.1a	Conduct library-dependent BST on 180 samples from the Lampasas River watershed and 180 samples from the Leon River watershed using ERIC-RP	AgriLife-EP	Nov 10	Jan 12
4.1b	Use Texas *E. coli* BST library to identify likely human and animal sources of *E. coli*	AgriLife-EP	Nov 10	Jan 12
4.2a	Isolate *E. coli* from 100 known source fecal samples received from AgriLife-TP	AgriLife-EP	Nov 10	Jan 12
4.2b	Screen approximately three isolates from each fecal sample using ERIC-PCR and select approximately 200 isolates for RiboPrinting and inclusion in the Texas *E. coli* BST library	AgriLife-EP	Nov 10	Jan 12
4.3	Develop technical reports for each watershed detailing BST results and present the results in Leon and Lampasas Rivers stakeholder meetings	AgriLife-EP	Feb 12	Jul 12

Section A7: Quality Objectives and Criteria for Data Quality

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

Following are actions that will be undertaken by this project to assess bacterial pollution within Lampasas and Leon Rivers Watershed:

- Monitor water quality in Lampasas and Leon Rivers by in-stream water sampling
- Collect known source fecal samples and incorporate them into the Texas *E. coli* BST Library
- Determine the sources of bacterial impairment using BST

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

When sufficient flow (above 7Q2 or 0.1 cfs) is present, routine grab samples will be collected on a monthly basis. During routine sampling measurements of dissolved oxygen (DO), conductivity, pH, salinity, stream flow (cfs), and water temperature will be obtained *in situ*. Water samples will be analyzed for *E. coli* concentrations.

Table A.7-1. Data Quality Objectives for Measurement Data

NA = Not applicable; mg/L = milligrams per liter; col = colonies; mL = milliliters; m/s = meters per second; µS/cm = microsiemens per centimeter; ft = feet; m = meters; °C = degrees Celsius

Parameter	Units	Method Type	Method	Method Description	Parameter Code	AWRL[1]	Precision of Laboratory Duplicates	Accuracy[2]	Percent Complete[3]
Flow Severity	1-no flow 2-low 3-normal 4-flood 5-high 6-dry	Visual Observation	TCEQ SOP V-1	Field observation	01351	NA	NA	NA	
Flow	cfs	Handheld meter	TCEQ SOP V-1	Automated Instrument	00061	NA	NA	NA	
Flow Method	1-gage 2-electric 3-mechanical 4-weir/flume 5-Doppler	Handheld meter	TCEQ SOP V-1	Automated Instrument and Calculation	89835	NA	NA	NA	
Water Temperature	˚C	Handheld meter	USEPA 170.2	Automated Instrument	00020	0.2	NA	±0.25	
Specific Conductance	µS/cm	Handheld meter	USEPA 120.1	Automated Instrument	00094	20 µS/cm	NA	±2% of range	
Dissolved Oxygen	mg/L (ppm)	Handheld meter	USEPA 360.1	Automated Instrument	00300	2.0	NA	±0.2	
pH	pH units	Handheld meter	USEPA 150.1	Automated Instrument	00400	0.2	NA	±0.2	

Table A.7-1. continued

Parameter	Units	Method Type	Method	Method Description	Parameter Code	AWRL [1]	Precision of Laboratory Duplicates	Accuracy[2]	Percent Complete [3]
E. coli in water	CFU/ 100 mL	Membrane filter culture on modified mTEC agar	USEPA 1603	Membrane Filter	31648	1	3.27* ΣRlog/n	NA	NA
E. coli RiboPrint fingerprint	NA	DNA/ image matching	AgriLife-EP SOP	RiboPrinting	NA	NA	85% identical[4]	90% correct[4]	90
E. coli ERIC-PCR fingerprint	NA	DNA/ image matching	AgriLife-EP SOP	ERIC-PCR	NA	NA	85% identical[4]	90% correct[4]	90

[1] minimum detection limits for field parameters represent manufacturer specifications and will be used for the AWRL in this instance.
[2] Manufacturer specifications are presented for accuracy limits and method detection limits for field parameters.
[3] The objective is for 90% of the data to be collected.
[4] Accuracy and laboratory method precision for BST will be determined using an *E. coli* QC isolate and *E. coli* from known-source samples

Ambient Water Reporting Limits (AWRLs)

The AWRL establishes the reporting specification at or below which data for a parameter must be reported based on given freshwater screening criteria. The AWRLs specified in Table A7.1 are the program-defined reporting specifications for each analyte and yield data of acceptable quality for 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 TSSWCB:

- 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 samples analyzed.

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

Precision

The precision of laboratory data is a measure of the reproducibility of a result from repeated analyses. It is strictly defined as a measure of the closeness with which multiple analyses of a given sample agree with each other. Precision is assessed by repeated analyses of a sample. For quantitative microbiological analyses, the method to be used for calculating precision is the one outlined in *Standard Methods for the Examination of Water and Wastewater,* 21st Edition, section 9020 B.8.b.

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

The relative percent deviation (RPD) $_{bacteria}$ should be lower than 3.27 ΣRlog/n, where Rlog is the difference in the natural log of duplicates for the first 15 positive samples.

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.

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

Accuracy

Accuracy is a statistical measurement of correctness and includes components of systemic error. A measurement is considered accurate when the result reported does not differ from the true situation. Performance limits for all measured parameters are specified in Table A.7-1.

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

Accuracy for BST methods will be assessed using ERIC-RP data for *E. coli* (QC101) and *E. coli* from known-source samples. For the *E. coli* methods the data quality objective is 90% accuracy for correct identification to library strain or source.

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 prepared with verified and known amounts of all target analytes in the sample matrix and by calculating percent recover. Results are compared against measurement performance specifications and used during evaluation of analytical performance.

Sensitivity

Sensitivity is a measure that is used to determine a method's detection limits. The detection limit of quantitative methods is defined as the minimum concentration of a substance that can be measured with a given level of confidence that the analyte concentration is greater than zero (*QA/QC Guidance for Laboratories Performing PCR Analyses on Environmental Samples* USEPA, 2004). For presence/absence methods, the detection limits the minimum concentration of analyte that produces a positive response with a given level of confidence. The detection limits can be expressed as the minimum number of organisms or of the target sequence copy number in a given volume. Many uncertainties can affect the detection limit; some are:

- The type of target nucleic acid being detected (e.g. DNA, mRNA, tRNA, etc.)
- The secondary structure and the GC content of the nucleic acid target molecule
- The matrix from which the organism is located
- The detection of microbes that are inactivated by physical and chemical disinfectants

For analyzing environmental samples using PCR, the detection limits can be for the limit of the entire method or the limit of the PCR procedure. For bacteria, protozoa, and fungi these limits are often measured in terms of the minimum detectable counts or CFUs.

Representativeness

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

Representativeness will be measured with the completion of sample collection in accordance with the approved QAPP.

Comparability

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

Completeness

The completeness of the data is a measure of how much of the data is available for use compared with the total potential data. Ideally, 100% of the data would be available. However, the possibility of unavailable data due to accidents, weather, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 90% data completion is achieved. Should less than 90% data completeness occur, the TWRI PM will initiate corrective action. Data completeness will be calculated as a percent value and evaluated with the following formula:

% completeness = (SV X 100) / ST

Where: SV = number of samples with a valid analytical report
ST = total number of samples collected

Section A8: Special Training Requirements/Certifications

Surface Water Quality Monitoring

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

Fecal Sample Collection

All personnel involved in collecting fecal samples have received the appropriate education and training required to adequately perform their duties. No special certifications are required. AgriLife-TP personnel have been briefed by AgriLife-EP personnel on appropriate techniques.

BST Analysis

All personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. AgriLife-TP and AgriLife-EP personnel involved in this project have been trained in the appropriate use of field equipment, laboratory equipment, laboratory safety, cryogenics safety, and all applicable SOPs where applicable. Each laboratory analyst must demonstrate their capability to conduct each test that the analyst performs to the Lab Director. This demonstration of capability is performed before analyzing samples and annually thereafter.

E. coli Enumeration

The AgriLife-TP laboratory is now certified for use of USEPA method 1603 granted by the National Environmental Laboratory Accreditation Conference (NELAC). This method is a quantitative method used to analyze *E. coli* and yields a direct count of bacteria in water based on the development of bacteria colonies that grow on the surface of the membrane filter. As a part of the NELAC approval process, the AgriLife-TP Lab had to select a stand-alone name for their facility. The name selected is the "*Water Science Laboratory*."

Section A9: Documentation and Records

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

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

Surface Water Quality Monitoring

Hard copies of general maintenance records, all field data sheets, chain of custody (COC) forms, laboratory data entry sheets, calibration logs, and corrective action reports (CARs) will be archived by AgriLife-TP for at least five years. In addition, AgriLife-TP will archive electronic forms of all project data for at least five years. All electronic data are backed up on an external hard drive monthly, compact disks weekly, and is simultaneously saved in an external network folder and the computer's hard drive. A blank CAR form is presented in Appendix A, a blank COC form is presented in Appendix B, and blank field data reporting forms are presented in Appendix C.

Fecal Sample Collection

Field data sheets, COCs and Sheets of Lading (Appendix D-5) will be filled out by AgriLife-TP and shipped along with fecal samples to AgriLife-EP. These documents will be kept on file by AgriLife-EP following their receipt.

BST Analysis

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

QAPP Revision and Amendments

Until the work described is completed, this QAPP shall be revised as necessary and reissued annually on the anniversary date, or revised and reissued within 120 days of significant changes, whichever is sooner. The last approved versions of QAPPs shall remain in effect until revised versions have been fully approved; the revision must be submitted to the TSSWCB for approval at least 60 days before the last approved version has expired. If the entire QAPP is current, valid, and accurately reflects the project goals and the organization's policy, the annual re-issuance may be done by a certification that the plan is current. This will be accomplished by submitting a cover letter stating the status of the QAPP and a copy of new, signed approval pages.

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

Section B1: Sampling Process Design (Experimental Design)

Surface Water Quality Monitoring

The primary goal of this project is to continue monitoring subwatersheds through data collection and analysis, and provide data to inform stakeholders of any potential or existing water quality issues and/or problems. In addition, water samples will be analyzed to determine the source of bacteria entering the stream. This information will be instrumental in the evaluation of potential BMP implementation and WPP development. Achievement of these objectives will support decisions on how to best target management measures for reduction of fecal bacteria levels in the Leon and Lampasas Rivers watersheds. The waterborne constituents that will be measured are shown in Table B1-1.

Table B1-1. Waterborne Constituents

Parameter	Status	Reporting Units
Laboratory Parameters		
Escherichia coli	Critical	cfu/100ml
Field Parameters		
Dissolved Oxygen	Non-critical	milligrams per liter (mg/L)
Potential Hydrogen (pH)	Non-critical	pH standard units
Specific Conductance	Non-critical	microsiemens per centimeter (µS/cm)
Water Temperature	Non-critical	degrees Celsius (°C)
Flow	Critical	cubic feet per second (cfs)
Flow Severity	Non-critical	1-no flow, 2-low, 3-normal, 4-flood, 5-high, 6-dry

The sampling program is designed to characterize water quality of both base and high flow conditions in the Leon and Lampasas Rivers watersheds. Water quality grab samples will be collected at monthly intervals for all constituents. Routine grab samples will be scheduled for collection on monthly basis but will only be taken if water is flowing at sampling sites. Sampling locations are described in Table B.1-2. Physical parameters that will be measured *in situ* during routine sampling and include flow rate, flow severity, water level, specific conductance, DO, pH and water temperature. Sites that are dry or with pooled water will not be sampled and will be noted on the field data sheet. Water quality samples collected as part of the routine sampling schedule will be analyzed for bacteria.

In order to obtain representative results, ambient water sampling will occur on a routine schedule over the course of 12 months, capturing dry and runoff-influenced events at their natural frequency. There will be no prejudice against rainfall or high flow events, except that the safety of the sampling crew will not be compromised in case of lightning or flooding; this is left up to the discretion of the sampling crew.

In the instance that a sampling (Table B.1-2.) site is inaccessible, no sample will be taken and will be documented in the field notebook. If, near the end of the study, the TSSWCB PM/QAO agrees that the sampling has not achieved good representativeness of typical conditions, the final sampling event(s) may be restricted to target a particular environmental condition (e.g., rainfall).

Table B.1-2. Tentative Leon River watershed BST sampling locations

RD= Road; CR= County Road; FM= Farm to Market Road; SH= State Highway

TCEQ Station_ID	Location	Latitude	Longitude
11934	LEON RIVER AT US 67/US 377	31.95778	-98.4593
17379	WALNUT CREEK AT FM 1476	31.97312	-98.4367
11933	LEON RIVER AT COMANCHE CR 382	31.82971	-98.2575
11818	INDIAN CREEK AT CR 304	31.88658	-98.4381
11808	RESLEY CREEK AT COMANCHE CR394	31.81303	-98.2240
11930	LEON RIVER AT CR 431	31.60882	-97.8968
11817	SOUTH LEON RIVER AT SH 36	31.84813	-98.3708
11929	LEON RIVER AT CORYELL CR 183	31.52514	-97.8601
17547	PECAN CREEK AT SH 22	31.71031	-98.0563
18405	PLUM CREEK AT CORYELL CR 106	31.5126	-97.9001
11932	LEON RIVER AT US 281	31.78746	-98.1211
11804	CORYELL CREEK AT FM 107	31.39278	-97.5994
17501	LEON RIVER AT FAUNT LEROY PARK (GATESVILLE)*	31.46250	-97.7492
11928	LEON RIVER AT US 84**	31.43503	-97.7595
11926	LEON RIVER AT SH 36	31.38369	-97.7017
11925	LEON RIVER AT FM 1829	31.33584	-97.6425

* Flow determined from upstream USGS gauge (TCEQ 11928) when stream cannot be safely waded.
**Alternate flow data station for TCEQ 17501 during periods of high flow. No other data used from this station.

Table B.1-3. Tentative Lampasas River watershed BST sampling locations

RD= Road; CR= County Road; FM= Farm to Market Road; SH= State Highway

TCEQ Station_ID	Location	Latitude	Longitude
15762	LAMPASAS RIVER AT US 84	31.48027	-98.2735
15770	LAMPASAS RIVER AT LAMPASAS CR 2925	31.37584	-98.1798
TBD	BENNETT CREEK AT LAMPASAS CR 2901	31.40775	-98.2395
16404	LAMPASAS RIVER AT FM 2313	31.11900	-98.0565
11872	SULPHUR CREEK AT NARUNA RD	31.05040	-98.1852
15781	SULPHUR CREEK AT LAMPASAS CR 3010	31.07091	-98.1353
TBD	MESQUITE CREEK AT LAMPASAS CR 4390	31.05357	-98.0487
11897	LAMPASAS RIVER AT US 190	31.08167	-98.0164
11724	ROCKY CREEK AT FM 963	30.98541	-97.9266
18759	REESE CREEK NR FM 2670 BR985	30.97930	-97.7847
11896	LAMPASAS RIVER AT SH 195	30.97248	-97.7786
TBD	CLEAR CREEK AT OAKALLA RD	31.00634	-97.8887
TBD	SCHOOL CREEK AT US 281	31.21433	-98.1731
15763	SIMMS CREEK AT US 281	31.26815	-98.1746
15250	SULPHUR CREEK AT LAMPASAS CR 3050	31.08544	-98.0507

Fecal Sample Collection

Collection of fecal material samples from known sources will be used to validate the BST methodologies. Approximately 100 known source samples from domestic animals, wildlife and human sources will be collected by AgriLife-TP throughout the course of the project. These known sources of bacteria will not be collected from the same locations during every sampling event due to the nature of the animals. Human sources are from specific areas, but will be selected based on cooperation of the individuals. Based on the inherent randomness of known source sampling, specific global positioning system (GPS) coordinates will be documented at the time of collection, but cannot be listed in advance for sample collections of this nature.

AgriLife-TP will coordinate with watershed coordinators funded through projects 06-12 and 07-11 to identify appropriate potential fecal sources to target within each watershed. Watershed coordinators will also help to identify volunteers who will allow access to the identified sources of fecal material.

Fecal sample collection will be conducted early on during the project to evaluate the use and accuracy of the Texas *E. coli* BST Library to identify fecal pollution sources. Depending on results, additional known source fecal samples may need to be collected (through TSSWCB project 10-50).

Section B2: Sampling Method Requirements

Surface Water Quality Monitoring

Typically, water samples will be collected directly from the stream (midway in the stream channel) into sterile wide-mouthed polypropylene bottles or bags. All sample containers will be labeled with the following information:

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

Care will be exercised to avoid the surface microlayer of water, which may be enriched with bacteria and not representative of the water column. In cases where, for safety reasons, it is inadvisable to enter the stream bed, and boat access is not practical or available, staff will use a clean bucket and rope from a bridge to collect the samples from the stream. If a bucket is used, care will be taken to avoid contaminating the sample. Specifically, technicians must exert care to ensure that the bucket and rope do not come into contact with the bridge. The bucket must be thoroughly rinsed between stations. Buckets are also to be sanitized between sampling stations with a bleach- or isopropyl alcohol-soaked wipe. The first bucketful of water collected from a bridge is used to rinse the bucket. Rinse water is not returned to the stream, but is instead disposed of away from the sampling site to ensure that the collected sample will not be affected by the bleach or alcohol residual. Samples are collected from subsequent buckets of water. This type of sampling will be noted in the field records.

Water temperature, stream flow, pH, specific conductivity, and DO will be measured and recorded *in situ* with a multiprobe whenever samples are collected. All field measurements will be conducted in accordance with the methods listed in Table B.4-1. Measurements will only be taken if water is flowing. If a site is not flowing but pooled or dry, that will be noted on the field data sheet. All samples will be transported in an iced container to the laboratory for analysis within required sample holding times.

Table B.2-1. Container Types, Preservation Requirements, Temperature, Sample Size, and Holding Time Requirements.

Parameter	Matrix	Container	Preservation	Temperature	Sample Size	Holding Time
E. coli	water	sterile plastic bag	none	4°C	125 ml	6 hours[1]
E. coli water and fecal isolates	NA-MUG agar	Petri dish 100mm x 15mm	20% glycerol; 80% tryptic soy broth	44.5°C	5 colony streaks	20 – 24 hrs, then frozen
Fecal specimen	feces	sterile plastic bottle placed in biohazard bag	none	4°C	0.5 g – 30 g	3 days

[1] 6 hours to deliver to laboratory. In the case that this 6-hour holding time is not met, the *E. coli* quantitative count will be flagged and not reported.

Documentation of Field Sampling Activities

Field sampling activities are documented on field data reporting forms as presented in Appendix C. Field observations (flow severity and days since last significant precipitation) are based on SOPs in the *TCEQ Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue* (October 2008). All sample information will be logged into a field log. The following will be recorded for all water sampling:

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

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

Recording Data

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

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

- Close-outs on incomplete pages with an initialed and dated diagonal line.

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

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

Fecal Sample Collection

Fecal samples will be obtained by collecting fecal samples from areas where animals were visually observed defecating by technician; i.e. deer or feral hogs at feeders; and samples collected from animals recently killed by cars (within 24 hours) or legally harvested by hunters who have agreed to work with the technician. Fecal samples from killed animals will be collected from approximately the last 1 foot of the large intestine as close to the rectum as possible. Only fecal material will be shipped to AgriLife-EP.

Documentation of Field Sampling Activities

All samples will be collected in approved specimen containers with spoon attached to the inside of the screw on lid or biohazard bag. Specimen container will be labeled with:

- Date
- Time
- Location
- Species
- Samplers initials

Safety is an issue when working with fecal samples due to the bacterial concentration. Hazardous material safety handling instructions will be included in a file for driver to carry that will be visible on seat or dash of vehicle in case of accident or being stopped by law enforcement officers. Biohazard signs will be placed on the cooler containing samples collected for transport to AgriLife-TP. Sheets of Lading (Appendix D-5) will be on hand with the field technician and completed for each fecal sample collected along with a COC form.

Section B3: Sample Handling and Custody Requirements

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. The COC form is used to document sample handling during transfer from the field to the laboratory and inter-laboratory. The sample number, location, date, changes in possession and other pertinent data will be recorded in indelible ink on the COC. The sample collector will sign the COC and transport it with the sample to the laboratory. At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. Sample numbers will then be recorded into a laboratory sample log, where the laboratory staff member who receives the sample will sign it. A copy of a blank COC form used on this project is included as Appendix B.

Sample Labeling

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

Sample Handling

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

Failures in Chain-of-Custody and Corrective Action

All failures associated with COC procedures are to be immediately reported to the TSSWCB PM. Failures include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The Project Leader and the TSSWCB PM/QAO will determine if the procedural violation may have compromised the validity of the resulting data. Any failure that potentially compromises data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB in the QPR. The CARs will be maintained by the TSSWCB PM.

Section B4: Analytical Methods Requirements

E. coli in water samples will be isolated and enumerated by laboratory personnel using modified mTEC agar, USEPA Method 1603 [*USEPA -821-R-06-011. July 2006. Escherichia coli in Water by Membrane Filtration Using Modified Membrane-Thermotolerant Escherichia coli (modified m-TEC) Agar*]. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate. The modified medium contains a chromogen, 5-bromo-6-chloro-3-indolyl-ß-D-glucuronide, which is catabolized to glucuronic acid and a red- or magenta-colored compound by *E. coli* that produce the enzyme ß-D-glucuronidase. This enzyme is the same enzyme tested for using the MUG substrate and UV fluorescence in other *E. coli* assays. A complete listing of methodology used to analyze water and fecal samples for bacteria is given in Appendix D.

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

Table B.4-1. Laboratory Analytical Methods

Parameter	Method	Equipment Used
Laboratory Parameters		
Escherichia coli	USEPA 1603	Filtration apparatus, incubator
E. coli RiboPrint fingerprint	AgriLife-EP SOP	RiboPrinter
E. coli ERIC-PCR fingerprint	AgriLife-EP SOP	PCR thermal cycler, gel electrophoresis apparatus
Field Parameters		
Dissolved Oxygen	USEPA 360.1	Hach Multiprobe
pH	USEPA 150.1	Hach Multiprobe
Specific Conductance	USEPA 120.1	Hach Multiprobe
Water Temperature	USEPA 170.2	Hach Multiprobe
Flow Severity	TCEQ SOP V-1	Field observation
Flow	TCEQ SOP V-1	OTT Current Meter Teledyne Acoustic Doppler Current Profiler

USEPA = Methods for Chemical Analysis of Water and Wastes, March 1983
SM = Standard Methods for Examination of Water and Wastewater, 21st edition
SOP = Standard Operating Procedure
USGS = Techniques of Water Resources Investigations, Book 3, Chapter A8, 1980

Section B5: Quality Control Requirements

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

Field Blanks
Field blanks consist of sterile distilled water that is taken to the field and transferred to the appropriate container in precisely the same manner as a field sample during the course of a sampling event. They are used to assess contamination from field sources such as airborne materials, carryover from prior sampling sites, and containers. A field blank should be included for each sampling event. The analysis of field blanks should yield a value of no colonies detected.

Laboratory Blanks
Laboratory blanks, or negative controls, consist of 100-ml aliquots of sterile distilled water that are processed in the same manner as a field sample, at the beginning and the end of a sample set. They are used to assess the sterilization techniques employed throughout the sample process. Laboratory blanks will be included at the beginning and the end of the sample set for each sampling event. The analysis of laboratory blanks should yield a value of no colonies detected. In addition, no template negative controls will be analyzed for each batch of ERIC-RP.

Field Splits
A field split is a single sample subdivided by field staff immediately following collection and submitted to the lab as two separately identified samples according to procedures specified in the TCEQ SOP. Split samples are preserved, handled, shipped, and analyzed identically and are used to assess variability in all of these processes. Field splits apply to conventional samples only. According to procedures specified in the TCEQ SOP, field splits are to be submitted with every tenth sample. If less than 10 samples are collected in a month, submit one set of splits per month.

The precision of field split results is calculated by RPD using the following equation:

$$RPD = (X1-X2)/((X1+X2)/2)$$

A 20% 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 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.

Positive Control

AgriLife-TP will purchase, store, and use "BioBalls" for positive controls. Each time a set of samples is run a positive control will be performed in the lab. This control should always be positive for *E. coli* after recommended incubation time. In addition, positive controls will be analyzed for each batch of *E. coli* ERIC-PCR and RiboPrinting samples.

Failures in Quality Control and Corrective Action

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

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

To minimize downtime of all measurement systems, spare parts for field and laboratory equipment will be kept in the laboratory, and all field measurement and sampling equipment, in addition to all laboratory equipment, must be maintained in a working condition. All field and laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in Standard Methods for the Examination of Water and Wastewater, 21st Edition. Maintenance and inspection logs will be kept on each piece of laboratory equipment and general maintenance checklists will be filled out for field sampling equipment, by the field technician, prior to each sampling event.

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

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

Table B.6-1. Equipment Inspection and Maintenance Requirements

Equipment	Relevant Testing, Inspection and Maintenance Requirement
D.O. meter	SM 9020 B 3.a
Conductivity meter	SM 2510 B 2.c
pH meter	SM 4500-H^+ B 2 b
Thermometers	SM 9020 B 3.a
Water deionization units	SM 9020 B 3.d
Media dispensing apparatus	SM 9020 B 3.f
Autoclaves	SM 9020 B 3.h
Refrigerator	SM 9020 B 3.i
Ultra Low Freezer	SM 9020 B 3.j
Membrane filter equipment	SM 9020 B 3.k
Ultraviolet sterilization lamps	SM 9020 B 3.l
Biological safety cabinet	SM 9020 B 3.m
Incubators	SM 9020 B 3.o
Glassware and plastic ware	SM 9020 B 4.a
Utensils and containers	SM 9020 B 4.b
Dilution water bottles	SM 9020 B 4.c
Flow Meter	Product Owner's Manual
RiboPrinter	Per manufacturer and annual preventative maintenance
PCR thermal cycler	Per manufacturer

Section B7: Instrument Calibration and Frequency

Surface Water Quality Monitoring

Field equipment calibration requirements are contained in the *TCEQ Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue* (October 2008). 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.

BST Analysis

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

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

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

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

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

Table B7.1. Instrument Calibration Requirements

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

Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

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

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

Surface Water Quality Monitoring

This QAPP does not include the use of routine data obtained from non-direct measurement sources. Only data collected directly under this QAPP is submitted to the SWQMIS database.

BST Analysis

Data analyzed using BST analysis methods for this project will consist largely of data produced *during the course of this study and will adhere to the guidance set forth in this QAPP*. Known source fecal samples previously collected and analyzed under other QAPPs such as the QAPP for TSSWCB project 06-11, *Watershed Protection Plan Development for Buck Creek,* and entered into the Texas *E. coli* BST Library will also be utilized as a cross reference and to see how the state-wide BST library compares to the Lampasas and Leon BST samples collected. All samples currently included in the Texas *E. coli* BST Library have been collected, processed and screened prior to their inclusion in the library.

Section B10: Data Management

Field Collection and Management of Routine Samples

Field staff will visit sampling sites on a monthly basis to collect grab water samples and measure field water quality parameters. Site identification, date, time, personnel, measurements of field parameters, and any comment concerning weather or conditions at the site are noted on a field data sheet. One field data sheet is filled out in the field for each site visited. An example of a field data sheet is shown in Appendix C. If no flow is observed at a site, samples will not be collected but information about the site visit will be recorded on the field data sheet and the site noted as pooled with no flow or dry. Information on the dates that sites were visited when no flow occurred will be recorded into a separate MS Excel workbook.

Field staff will measure DO, pH, water temperature and specific conductance at each stream site, using calibrated multi-sonde equipment. Flow rate will be recorded using an acoustic doppler flow meter. Measurements read from the instruments will be recorded on the field data sheet. Grab samples will be collected at the site, and an identification number (either a sample identification number or a site code) will be written in permanent marker on the outside of the sterile polypropylene sample bags.

Site codes are marked on sample bags in the field. The COC forms will be used if the collecting technician is in fact not the same person receiving samples into the lab. Site name, time of collection, comments, and other pertinent data are copied from the field data sheets to the COC.

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

Original data recorded on paper files will be stored for at least five years in a locked, restricted-access, fire-resistant storage area. Electronic data files will be archived to CD-ROM after approximately one year, and maintained in the fire-resistant storage area.

Laboratory Data

All field samples will be logged upon receipt, COC forms (if applicable) will be checked for number of samples, proper and exact I.D. number, signatures, dates, and type of analysis specified. The TSSWCB will be notified if any discrepancy is found and laboratory analysis will not occur until proper corrections are made. All samples will be stored at 4ºC until analysis. Bacteriological samples will be given a unique identification number and logged into the Microsoft Excel based database used to store field data. All backup and safety features of this database are the same as explained above. Enumerated bacteriological data will be manually entered into the database system for electronic storage. At least 10% of all data manually entered in the database will be reviewed for accuracy by the Project Leader to ensure that there are no

transcription errors. Hard copies of data will be printed and housed in the AgriLife-TP laboratory for a period of five years. Any COC's and bacteriological records related to QA/QC of bacteriological procedures will be housed at AgriLife-TP.

Sample Delivery to Other Laboratories
Water and fecal samples collected for BST analysis are collected and logged using the procedures described above in the field collection and lab data sections. The AgriLife-TP Research Technician(s) ensures that these samples are handled according to procedures laid out in this QAPP and that COC forms are correctly filled out for sample delivery to the AgriLife-EP lab. The AgriLife-TP Research Technician ships the samples, the appropriate Sheets of Lading for Fecal Specimen Transport (Appendix D-5) and COC forms to the AgriLife-EP lab via FedEx or other carrier in an appropriately labeled container that maintains appropriate sample temperatures with the use of dry ice. Once the samples are received at the AgriLife-EP lab, the COC forms are updated and the AgriLife-TP Research Technician is notified of the samples receipt.

Data Reporting
Data transmission between labs (AgriLife-EP to AgriLife-TP and AgriLife-TP to TWRI) occurs electronically. In the event that data files are too large to send via Email, a copy of the data set is copied to a CD-RW disc and mailed to the appropriate party. Data are recorded in Microsoft Excel format and submitted to the respective entity. AgriLife-TP maintains the project database and follows the guidelines listed above in protecting the data from corruption or loss.

Data will be reported according to the standards of the TSSWCB. Data intended to be submitted by TSSWCB to TCEQ for inclusion in SWQMIS for use in 305(b) assessments will be reported in a format consistent with *TCEQ Surface Water Quality Monitoring Data Management Reference Guide* (TCEQ 2010).

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

Section C1: Assessments and Response Actions

The following table presents the types of assessments and response action for activities applicable to this QAPP.

Table C.1-1. Assessments and Response Actions

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TWRI and AgriLife-TP	Monitoring of the project status and records to ensure requirements are being fulfilled. Monitoring and review of contract laboratory performance and data quality	AgriLife-TP and TWRI will report to TSSWCB PM via QPR.
Laboratory Inspections	Once per life of project (each lab)	TSSWCB QAO	Analytical and QC procedures employed at the laboratory	AgriLife-TP and AgriLife-EP have 45 days to respond in writing to the TSSWCB QAO to address corrective actions
Monitoring Systems Audit	Once per life of project	TSSWCB QAO	The assessment will be tailored in accordance with objectives needed to assure compliance with the QAPP. Field sampling, handling and measurement; facility review; and data management as they relate to the project	AgriLife-TP has 45 days to respond in writing to the TSSWCB QAO to address corrective actions

Corrective Action

The TWRI PM is responsible for implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the TSSWCB QAO.

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.

Section C2: Reports to Management

QPRs will be generated by TWRI and will note activities conducted in connection with the water quality monitoring program, items or areas identified as potential problems, and any variation or supplement to the QAPP. CARs will be utilized when necessary (Appendix A) and will be maintained in an accessible location for reference at AgriLife-TP. CARs that result in changes or variations from the QAPP will be made known to pertinent project personnel, documented in an update or amendment to the QAPP and distributed to personnel listed in Section A3.

Section D1: Data Review, Validation, and Verification

All data obtained from field and laboratory measurements will be reviewed and verified for integrity, continuity, reasonableness, and conformance to project requirements, and then validated against the DQOs outlined in Section A7. Only those data that are supported by appropriate QC data and meet the DQOs defined for this project will be considered acceptable for use.

The procedures for verification and validation of data are described in Section D2, below. AgriLife-TP and AgriLife-EP are responsible for ensuring that field and laboratory data collected are properly reviewed, verified, and submitted in the required format for the project database. TWRI is responsible for validating that all data collected meet the DQOs of the project and are suitable for submission to TSSWCB.

Section D2: Validation and Verification Methods

All data will be verified to ensure they are representative of the samples analyzed and locations where measurements were made, and that the data and associated QC data conform to project specifications. The Project Leader is responsible for the integrity, validation, and verification of the data each field and laboratory task generates or handles throughout each process. The field and laboratory QA tasks ensure the verification of field data, electronically generated data, and data on COC forms and hard copy output from instruments.

Verification, validation, and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified are evaluated against project specifications (Section A7 and Section B5) and are checked to ensure the verification of raw data for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data, or identified using computer-based statistical software such as SAS. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues that can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TSSWCB QAO to establish the appropriate course of action, or the data associated with the issue are rejected.

The AgriLife-TP and AgriLife-EP Project Co-Leaders, along with TWRI, are responsible for validating that the verified data are scientifically sound, defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to the TSSWCB. One element of the validation process involves evaluating the data for anomalies. The AgriLife-TP and AgriLife-EP Project Co-Leaders may designate other experienced water quality experts familiar with the waterbodies under investigation to perform this evaluation. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data, before data validation can be completed.

Table D.2-1. Data Review, Verification, and Validation Procedures

Data to be Verified	Field[†] Supervisor	Laboratory Supervisor	PM/QAO Task[‡]
Collection and analysis techniques consistent with SOPs and QAPP	X	X	X
Field QC samples collected for all parameters as prescribed in the QAPP	X		X
Field documentation complete	X		X
Instrument calibration data complete	X	X	X
Sample documentation complete	X	X	X
Field QC results within acceptance limits	X		X
Sample identifications	X	X	X
COC complete/acceptable	X	X	X
Sample preservation and handling	X	X	X
Holding times	X	X	X
Instrument calibration data	X	X	X
QC samples analyzed at required frequencies		X	X
QC samples within acceptance limits		X	X
Instrument readings/printouts	X	X	X
Calculations	X	X	X
Laboratory data verification for integrity, precision, accuracy, and validation		X	X
Laboratory data reports		X	X
Data entered in required format	X	X	X
Site ID number assigned	X		X
Valid STORET codes			X
Absence of transcription error	X	X	X
Reasonableness of data	X	X	X
Electronic submittal errors	X	X	X
Sampling and analytical data gaps	X	X	X

[†] Field and Laboratory Supervisor may be the same person for AgriLife-TP
[‡] TSSWCB PM / QAO will monitor data for QA/QC purposes as needed.
All other entities are required to inspect 100% of the data prior to approval

Section D3: Reconciliation with User Requirements

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

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only BST data that has met the data quality objectives described in this QAPP will be reported and included in the final project report. 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. Data and information produced thru this project will provide needed information pertaining to watershed characteristics, potential sources of pollution and will aid in the selection of BMPs to address identified water quality issues. Stakeholders will then be able to use this information in the development of comprehensive WPPs for their respective watersheds under TSSWCB projects 07-11 for the Lampasas River and 06-12 for the Leon River.

References

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.

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Appendix A: Corrective Action Report

Corrective Action Report
CAR #:______________

Date:____________________ Area/Location:_____________________

Reported by:____________________ Activity:__________________________

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

Possible causes:
____
____
____

Recommended corrective action:
____
____
____

CAR routed to:________________________________
Received by:__________________________________

Corrective Actions taken:
____
____
____
____

Has problem been corrected?: YES NO

Immediate Supervisor:_______________________________

Project Leader:__________________________________

Quality Assurance Officer:___________________________

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Appendix B: Chain of Custody Record

CHAIN OF CUSTODY RECORD

Project: *Leon/Lampasas BST*	**Remarks:** **AgriLife-TP Lab to AgriLife-EP Lab**
Name and signature of collector:	**Air bill #**

Station ID	Sample ID	Media Code	Sample Type	Preservative	Collection Date	Time

Relinquished by Agrilife-TP Lab Tech:	Date:	Time:	Received for AgriLife-EP lab by:	Date:	Time:

Laboratory Notes:

Media Code: (**FW**) Fecal Isolate from Water Sample; (**FF**) Fecal isolate from Feces; (**FS**) Fecal Sample; (**SS**) Sewage Sample

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Appendix C: Field Data Reporting Form

Field Data Reporting Form

Station ID | **Date:** mm/dd/yy | **Time:** 24:00 | **Depth (inches)**

Sample ID | **COC Number** | **Air Temp** . °F

Collector's Last Name(s)

AgriLife-TP

Collecting Agency

Station Description

Circle one:

Flow Severity: 1-no flow 2-low 3-normal 4-flood 5-high 6-dry

Days since last significant rainfall:

Weather Observations (use codes from back)

Dissolved Oxygen (DO) . mg/l

pH .

Specific Conductance , µS/cm

Water Temperature . °C

Flow Rate . (cfs)

Salinity . ppt

Type of sample collected: circle appropriate sample **Water** **Fecal** **Number:** ____

Other Observations:

Weather Observation Codes:

00: No significant weather observed
01: Clouds generally dissolving or becoming less developed
02: State of sky on the whole unchanged during the past hour
03: Clouds generally forming or developing during the past hour
04: Haze, smoke, or dust in suspension in the air, visibility equal to or greater than 1km
05: Smoke
10: Mist
12: Distant lightning
18: Squalls
20: Fog during previous hour,
21: Precipitation during previous hour
22: Drizzle (not freezing) or snow grains during previous hour
23: Rain (not freezing) during previous hour
25: Freezing drizzle or freezing rain during previous hour,
26: Thunderstorm (with or without precipitation) during previous hour,
27: Blowing or drifting snow or sand
28: Blowing or drifting snow or sand, visibility equal to or greater than 1 km
29: Blowing or drifting snow or sand, visibility less than 1 km
30: Fog
31: Fog or ice fog in patches
32: Fog or ice fog, has become thinner during the past hour
33: Fog or ice fog, no appreciable change during the past hour
34: Fog or ice fog, has begun or become thicker during the past hour
35: Fog, depositing rime
40: Precipitation
41: Precipitation, slight or moderate
42: Precipitation, heavy
43: Liquid precipitation, slight or moderate
44: Liquid precipitation, heavy
45: Solid precipitation, slight or moderate
46: Solid precipitation, heavy
50: Drizzle
51: Drizzle, not freezing, slight
52: Drizzle, not freezing, moderate
53: Drizzle, not freezing, heavy
54: Drizzle, freezing, slight
55: Drizzle, freezing, moderate
56: Drizzle, freezing, heavy
57: Drizzle and rain, slight
58: Drizzle and rain, moderate or heavy
60: Rain
61: Rain, not freezing, slight
62: Rain, not freezing, moderate
63: Rain, not freezing, heavy
64: Rain, freezing, slight
65: Rain, freezing, moderate
66: Rain, freezing, heavy
67: Rain or drizzle and snow, slight
68: Rain or drizzle and snow, moderate or heavy
80: Showers or intermittent precipitation
81: Rain showers or intermittent rain, slight
82: Rain showers or intermittent rain, moderate
83: Rain showers or intermittent rain, heavy
84: Rain showers or intermittent rain, violent
90: Thunderstorm
91: Thunderstorm, slight or moderate, with no precipitation
92: Thunderstorm, slight or moderate, with rain showers and/or snow showers
93: Thunderstorm, slight or moderate, with hail
94: Thunderstorm, heavy, with no precipitation
95: Thunderstorm, heavy, with rain showers and/or snow
96: Thunderstorm, heavy, with hail

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Appendix D
Standard Operating Procedures

D-1: Laboratory Protocol for Isolation and Confirmation of *Escherichia coli* from Fecal Specimen 64
D-2: Archival of *Escherichia coli* Isolates....65
D-3: ERIC-PCR of *Escherichia coli*....66
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D-5: Sheets of Lading for Fecal Specimen Transport 86

D-1: Laboratory Protocol for Isolation and Confirmation of *Escherichia coli* from Fecal Specimens

Note: All collection and handling of fecal specimens should be performed using protective gear (i.e. latex or nitrile gloves). Specimens should be handled aseptically to ensure sample quality and minimize exposure of personnel to pathogens. All feces collected will be placed in screw capped sterile containers. Containers will be labeled with: Name of collector, date, species, GPS location, and photo of specimen before collection. Containers will then be placed in ziplock biohazard bags with lading pouch. Information will be written on lading report and placed in the bag. Fecal specimens will be placed in an insulated cooler on ice during transport to the AgriLife-TP lab. All fecals should be cultured within 24 hours of reaching the lab.

Note: All handling of fecal specimens and cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens.

1. Using a bacteriological loop, streak a loop full of fecal material onto a labeled modified mTEC agar plate (EPA-821-R-06-011, Modified USEPA Method 1603; http://water.epa.gov/scitech/swguidance/methods/bioindicators/upload/2008_11_25_methods_method_biological_1603.pdf) for isolation of *E. coli* colonies.

2. Incubate the plate inverted at 44.5±0.2°C for 20 to 24 h.

3. Examine the plate for presumptive *E. coli* colonies, which will appear red or magenta colored.

4. Select up to three presumptive *E. coli* colonies and streak each colony for purity onto a labeled nutrient agar MUG (NA-MUG) plate.

5. Invert and incubate plates at 35 to 37°C for 20 to 24 h.

6. Examine the cultures using a **long-wave handheld UV lamp**. If there is a mixture of fluorescent and non-fluorescent colonies, select a well isolated fluorescent colony and streak again onto NA-MUG for purity.

7. At the discretion of the laboratory, additional biochemical tests such as urease, indole, and citrate tests may be performed.

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.

D-2: 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.

D-3: 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. <u>One full PCR batch on the MJ Research Cycler 48 well-plate will have 46 samples, *E. coli* QC101, and a no template control.</u>

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

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 in cold room (4°C) 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)

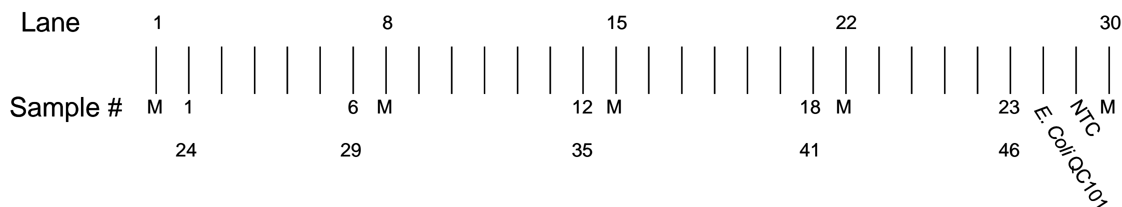
    f. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    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 Logic 200 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.

D-4: 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

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

2. 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.

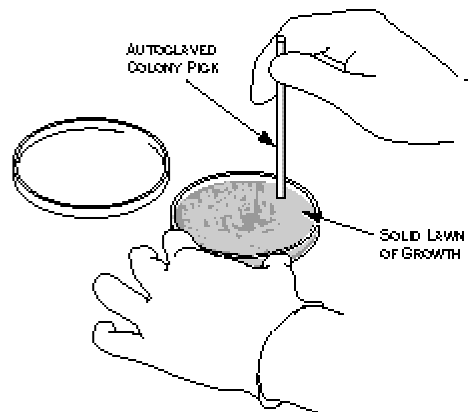
2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.

   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 µL 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.

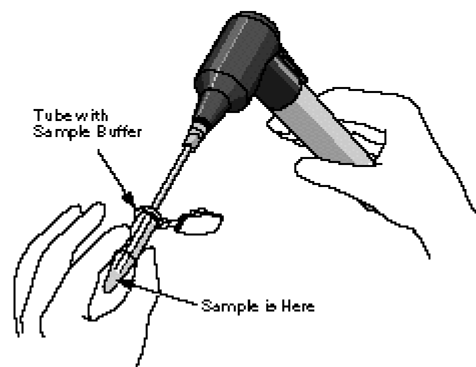
3. Press the ON lever on the mixer for about 5 seconds.

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

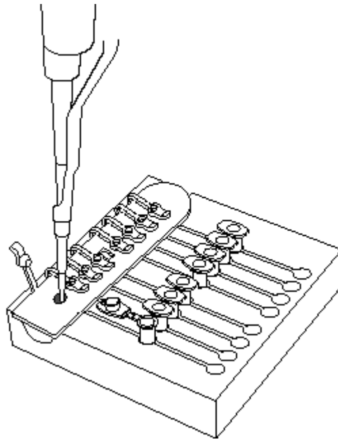
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.

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 µL pipetter, pipette 30 µL of sample from the microcentrifuge tube into the well.

3. Close the lid cover for the well.

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

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

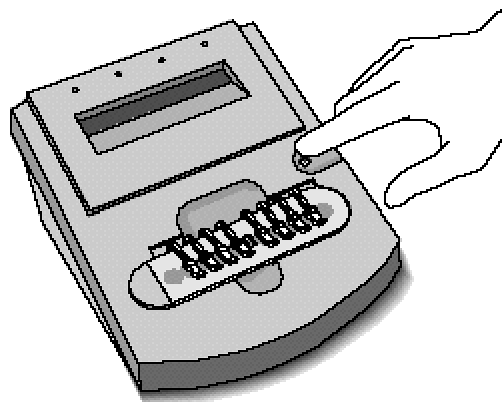
6. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).

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

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

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

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



2. Press the button on the Heat Treatment Station.

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

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

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

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

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

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

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

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

Creating and Loading a Batch

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

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

You can also create special batches:

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

From the Instrument Control Base Window:

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

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

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

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

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

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

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

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

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

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

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

10. Repeat for the other seven samples.

11. Click on Save and Submit Batch to Instrument.

Loading Disposables

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

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

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

1. Check the DNA Preparation Waste Container

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

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

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

2. Load the Sample Carrier

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

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

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

3. Load the DNA Prep Carrier

1. Remove the DNA Prep carrier from the refrigerator.

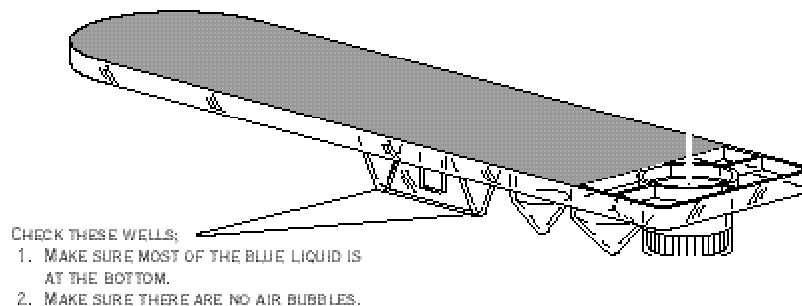
2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**

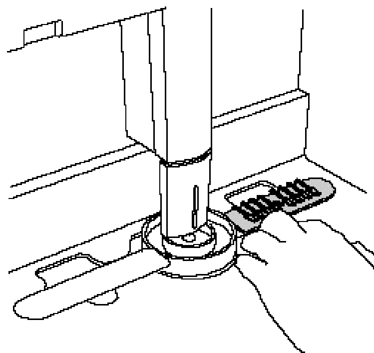
3. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.

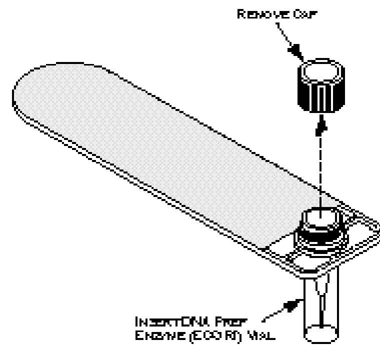
4. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as a 50 U/µL working stock as follows.**

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

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





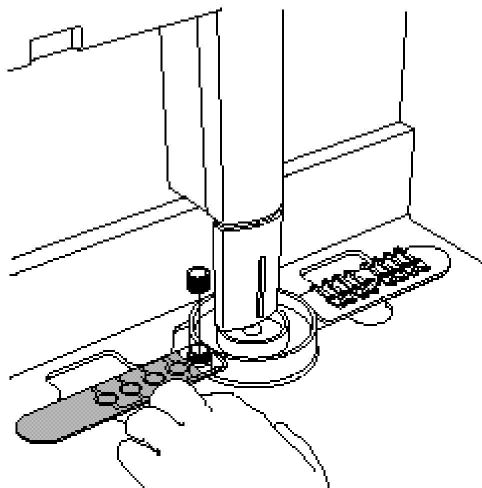


5. Remove the cap from the Enzyme vial.

6. Insert the vial into the carrier.

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

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

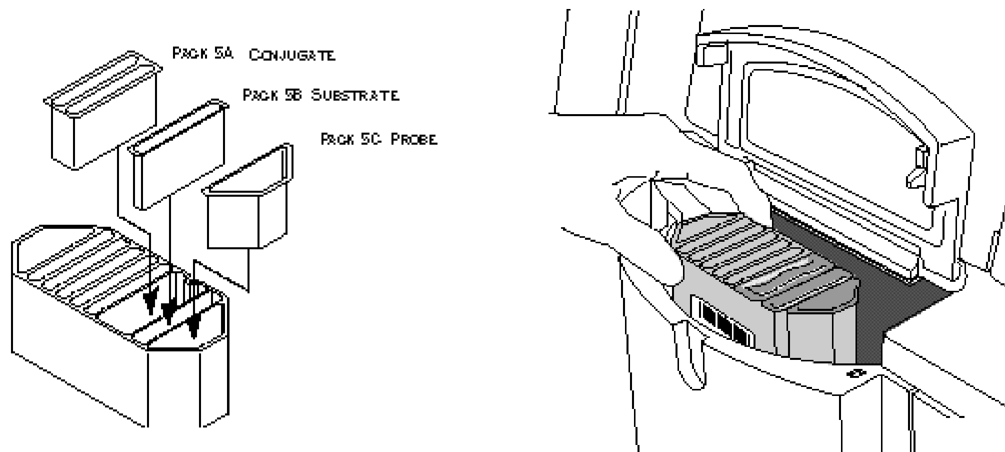


4. Load the MP Base and Carousel

1. Unpack the disposables.

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

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



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

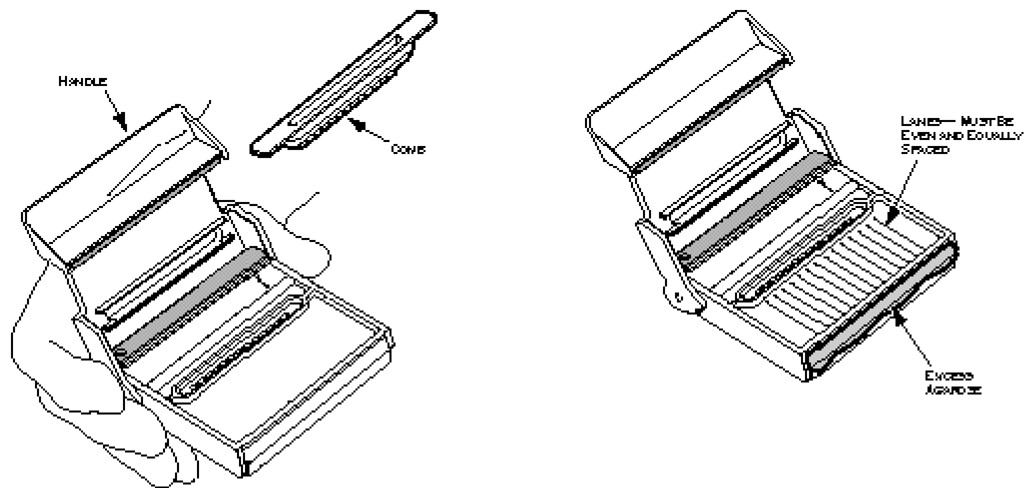
5. Load the Gel Cassette

1. Remove the gel cassette from its package.

2. Grasp one end of the rubber comb and gently pull the comb from the cassette.

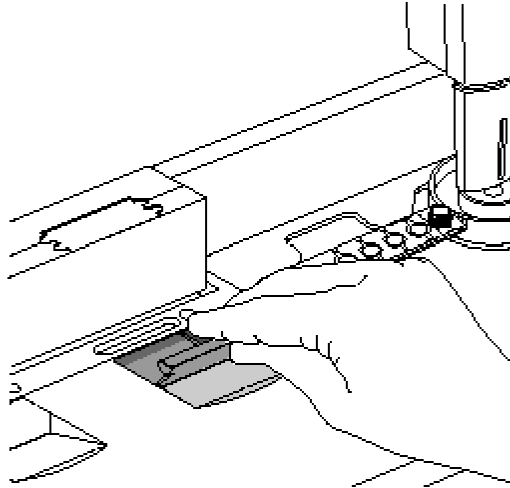
3. Unfold the handle of the cassette towards you until the handle snaps into place.

4. Check the front edge of the gel cassette and the lanes of the gel.



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

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

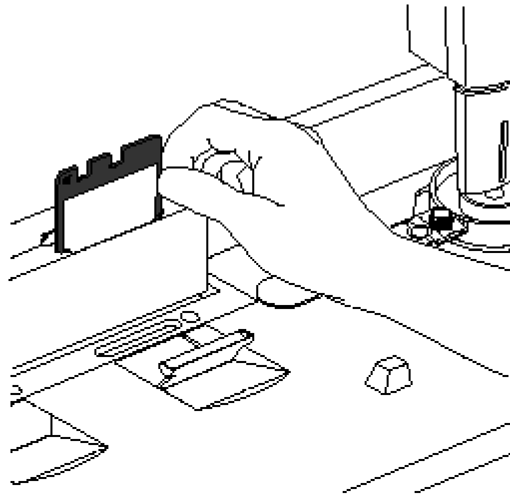


6. Press the cassette forward firmly until it snaps into place.

6. Load the Membrane

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

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



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

8. Load the Next Batch

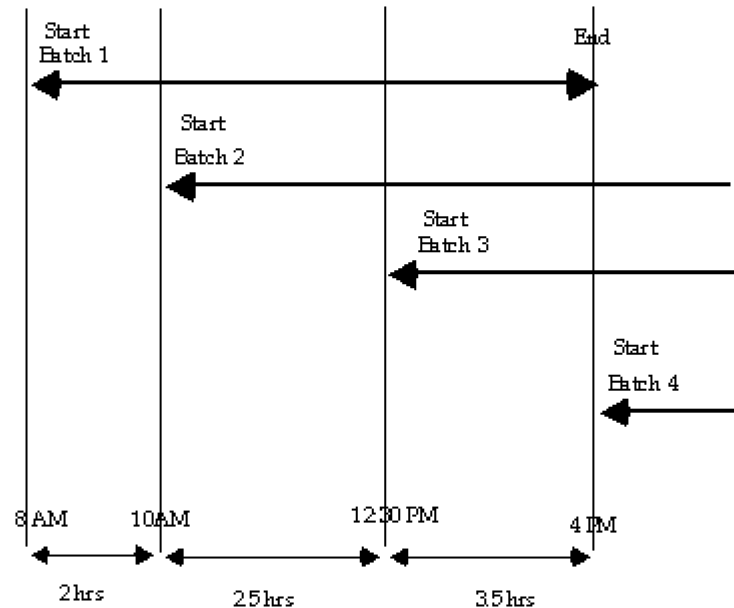
The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.
The chart above shows the approximate loading times for each batch in a work shift using only the VCA protocol.

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

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

Batch Report

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



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D-5: Sheets of Lading for Fecal Specimen Transport

Texas AgriLife Research and Extension Center
Temple, Texas
Project NameCollector:
Telephone #

In case of EMERGENCY:
Jodi Thomas: (254) 774-6006

Date:____________Time:______________

Sample: Fecal **Hazard:** Bacteria

Species:______________________________

Photo: **Yes** **No**

GPS: Lat______________Long____________

Technician:___________________________

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Appendix E

Sample Handling and Shipping for EPA Method 1603

Shipping and Handling of modified mTEC plates from EPA Method 1603

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

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

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

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

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

6. Notification of shipment should be sent to AgriLife EP via email, jatruesdale@ag.tamu.edu or phone, AgriLife EP 915-859-9111, no later than the day of overnight shipping. Notification should include *E. coli* count datasheet, tracking number, and direct AgriLife TP Lab contact person for confirmation upon receipt of samples.