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

Monitoring and Educational Programs Focused on Escherichia coli Bacteria and Nutrient Runoff on Dairy Operations in the Leon Watershed

TSSWCB Project Number 06-07
Revision #1

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

Prepared By:

Texas AgriLife Extension Service

Effective Period: Upon EPA Approval for 36 months,
with annual updates required

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

Dr. Sam Feagley
Texas AgriLife Extension Service
2474 TAMU
College Station, Texas 77843-2474
(979) 845-1460
s-feagley@ag.tamu.edu

A1. APPROVAL PAGE

Quality Assurance Project Plan for *Monitoring and Educational Programs Focused on Escherichia coli Bacteria and Nutrient Runoff on Dairy Operations in the Leon Watershed*

This page will be signed by all participants who will then sign a letter to document adherence to the QAPP (Appendix J).

United States Environmental Protection Agency (EPA), Region 6

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

Signature:_____ Date:_____

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

Signature:_____ Date:_____

Texas State Soil and Water Conservation Board (TSSWCB)

Name: Pamela Casebolt
Title: TSSWCB Project Manager

Signature:_____ Date:_____

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

Signature:_____ Date:_____

Texas AgriLife Extension Service (AgriLife Extension)

Name: Sam Feagley
Title: Project Manager/QAO/Data Manager

Signature:_____ Date:_____

Name: Diane Boellstorff
Title: Water Quality State Specialist

Signature:_____ Date:_____

Name: Ellen Jordan
Title: Dairy Specialist

Signature:_____ Date:_____

Name: Robert Scott
Title: County Extension Agent

Signature:_____ Date:_____

Name: Whit Weems
Title: County Extension Agent

Signature:_____ Date:_____

Texas AgriLife Research (AgriLife Research)

Name: George Di Giovanni
Title: Project Microbiologist

Signature:_____ Date:_____

Name: Terry Gentry
Title: Project Microbiologist

Signature:_____ Date:_____

Name: Jeff Brady
Title: Research Scientist

Signature:_____Date:_____

Name: Nichole Cherry
Title: Research Assistant/ Lab Technician

Signature:_____Date:_____

Name: Todd Bilby
Title: Extension Dairy Specialist

Signature:_____Date:_____

Administration

Name: Travis Miller
Title: Soil and Crop Sciences, Associate Dept. Head

Signature:_____Date:_____

Name: David Baltensperger
Title: Soil and Crop Sciences, Dept. Head

Signature:_____Date:_____

Name: Ed Smith
Title: Director, Texas AgriLife Extension Service

Signature:_____Date:_____

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LIST OF ACRONYMS

AWRL	**Ambient Water Reporting Limit**
BMP	**Best Management Practices**
CAR	**Corrective Action Report**
CEA	**County Extension Agent**
CFU	**Colony Forming Units of Bacteria**
COC	**Chain-of Custody**
CNMP	**Comprehensive Nutrient Management Plan**
DNA	**Deoxyribonucleic Acid**
DQO	**Data Quality Objective**
EC	**Electrical Conductivity**
EOF	**Edge-of-field**
EP AREC	**Texas A&M El Paso Agricultural Research and Extension Center**
ERIC-PCR	**Enterobacterial Repetitive Intergenic Consensus Sequence—Polymerase Chain Reaction**
FY	**Fiscal Year**
ICP	**Inductively Coupled Plasma**
LCS	**Laboratory Control Standard**
LMU	**Land Management Unit**
LOQ	**Limit of Quantitation**
NCCLS	**National Committee for Clinical Laboratory Standards**
NIST	**National Institute for Standards and Technology**
NO_3-N	**Nitrate Nitrogen**
NPS	**Nonpoint Source**
PCR	**Polymerase Chain Reaction**
PFGE	**Pulsed-field Gel Electrophoresis**
QA	**Quality Assurance**
QAO	**Quality Assurance Officer**
QAPP	**Quality Assurance Project Plan**
QC	**Quality Control**
RPD	**Relative Percent Deviation**
RL	**Reporting Limit**
SM	**Standard Methods for the Examination of Water and Wastewater**
SOP	**Standard Operating Procedure**
TCEQ	**Texas Commission on Environmental Quality**
TMDL	**Total Maximum Daily Load**
TP	**Total Phosphorus**
TSSWCB	**Texas State Soil and Water Conservation Board**
USEPA	**United States Environmental Protection Agency**

A3. DISTRIBUTION LIST

U.S. Environmental Protection Agency (USEPA) Region 6
1445 Ross Avenue, Suite 1200;
Dallas, Texas 75202-2733

Name: Henry Brewer;
Title: Texas NPS Project Manager, Water Quality Division

Texas State Soil and Water Conservation Board (TSSWCB)
P.O. Box 658;
Temple, Texas 76503

Name: Pamela Casebolt;
Title: TSSWCB Project Manager

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

Department of Soil & Crop Sciences
370 Olsen Blvd;
College Station, TX 77843-2474

Name: Sam Feagley; Ph.D.
Title: AgriLife Extension – Soil Environmental Specialist

Name: Diane Boellstorff; Ph.D.
Title: Texas AgriLife Extension Service –Water Quality Specialist

Name: Terry Gentry; Ph.D.
Title: AgriLife Research – Soil and Aquatic Microbiologist

Texas AgriLife Extension Service
17360 Coit Road;
Dallas, TX 75252

Name: Ellen Jordan
Title: Dairy Specialist

Texas AgriLife Research – El Paso
1380 A&M Circle;
El Paso, TX 79927

Name: George Di Giovanni; Ph.D.
Title: Environmental Microbiologist

Texas AgriLife Research
1229 North U.S. Hwy 281;
Stephenville, TX 76401

Name: Jeff Brady
Title: Research Scientist

Name: Nichole Cherry
Title: Research Assistant/ Laboratory Technician

Name: Todd Bilby
Title: Extension Dairy Specialist

A4. PROJECT/TASK ORGANIZATION

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

U.S. ENVIRONMENTAL PROTECTION AGENCY REGION 6

Henry Brewer, EPA Project Officer

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

TEXAS STATE SOIL WATER CONSERVATION BOARD

Pamela Casebolt, TSSWCB Project Manager

Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Provides the primary point of contact between AgriLife Extension and the TSSWCB. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified in the contract. Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants. Responsible for verifying that the QAPP is followed by AgriLife Extesnion. Notifies the TSSWCB QAO of significant project nonconformances and corrective actions taken as documented in quarterly progress reports from AgriLife Extension Project Manager.

Donna Long, TSSWCB Quality Assurance Officer

Coordinates reviews and approves QAPP and any amendments or revisions. Conveys QA problems to appropriate TSSWCB management. Monitors implementation of corrective actions. Coordinates and conducts audits of field and laboratory systems and procedures.

TEXAS AGRILIFE EXTENSION SERVICE

Sam Feagley, Project Manager/QAO/Data Manager

Responsible for implementing and monitoring TSSWCB requirements in contracts, QAPP, and QAPP amendments and appendices. Ensures monitoring systems audits are conducted to ensure QAPP is followed by AgriLife Extension participants and that projects are producing data of known quality. Ensures that subcontractors are qualified to perform contracted work. Ensures TSSWCB project manager and/or QAO are notified of deficiencies and nonconformance, and that issues are resolved. Responsible for managing data, validating that data, and ensuring that data collected are acceptable for reporting to TSSWCB. Responsible for supervising sampling and oversight of project activities. Responsible for field scheduling, staffing, and ensuring that staff is appropriately trained. Responsible for compiling all reports prior to submission to TSSWCB. Cooperates with AgriLife Extension participants for data interpretations, information distribution, manuscript development, and quarterly and final report development.

Diane Boellstorff, Texas AgriLife Extension, Water Quality Specialist

Responsible for providing field help for conducting rainfall simulations and runoff collection. Cooperates with project manager for data interpretations, information distribution, manuscript development and quarterly and final report development.

Ellen Jordan, AgriLife Extension Dairy Specialist

Responsible for assisting with the coordination of wastewater/manure sampling and interpretation of data. Cooperates with project manager for data interpretations, information distribution, manuscript development and quarterly and final report development.

TEXAS AGRILIFE RESEARCH

Terry Gentry, AgriLife Research, Soil & Crop Sciences – Soil & Aquatic Microbiology Specialist

Responsible for isolation, confirmation, ERIC-PCR fingerprinting, and archival of *E. coli* isolates. Will provide selected isolates to Dr. Di Giovanni for RiboPrinting. Responsible for laboratory training, supervision, and technical direction for *E. coli* isolation, archival, and ERIC-PCR fingerprinting.

George Di Giovanni, AgriLife Research- El Paso - Microbial Analysis

Responsible for isolation and purification of *E. coli* from manure, wastewater, and water samples, archival of *E. coli* cultures, ERIC-PCR screening and ribotyping of *E. coli* isolates, and data analysis. Responsible for laboratory supervision and technical direction for *E. coli* isolation and archival, and ERIC-PCR screening and ribotyping of *E. coli* isolates. Responsible for verifying that the QAPP is distributed and followed by the EP AREC laboratory, and that the data produced is of known and acceptable quality. Responsible for ensuring adequate training and supervision of all activities involved in generating analytical data by the EP AREC laboratory.

Jeff Brady, AgriLife Extension- Stephenville - Research Scientist

Responsible for overseeing collection of manure and wastewater from dairy operations, and for collection of stream samples. Will assist with and oversee analyses conducted at the Stephenville Research and Extension Center. Cooperates with project manager for data interpretations, information distribution, manuscript development and quarterly and final report development.

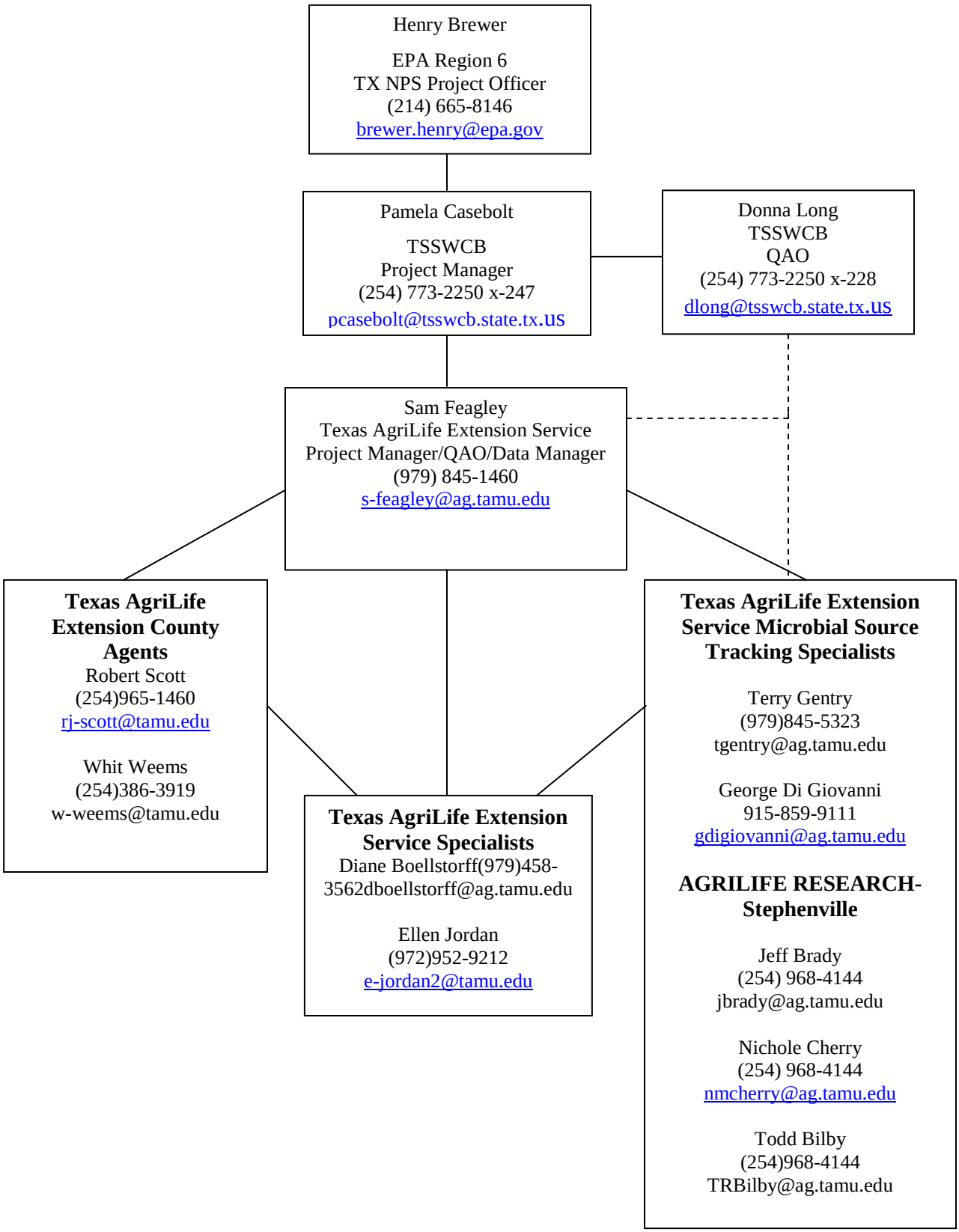
Nichole Cherry, AgriLife Extension- Stephenville – Research Technician

Responsible for coordinating collection of manure and wastewater from dairy operations, and for collection of stream samples. Will conduct /oversee analyses conducted at the Stephenville Research and Extension Center.

Todd Bilby, Texas AgriLife Extension Service, Stephenville – Diary Specialist

Responsible for overseeing collection of manure and wastewater from dairy operations, and for collection of stream samples. Will assist with and oversee analyses conducted at the Stephenville Research and Extension Center. Cooperates with project manager for data interpretations, information distribution, manuscript development and quarterly and final report development.

Figure A.4-1. Organization flow chart



A5. PROBLEM DEFINITION/BACKGROUND

In 2002, the Leon River below Lake Proctor was listed as being impaired for bacteria according to the Texas Water Quality and 303 (d) lists. Due to the listing for impairment, the Leon River Watershed was selected by the Texas Commission on Environmental Quality (TCEQ) for the development of a TMDL. As of January 2006, the TMDL was in the developmental process. Part of the TMDL development includes modeling the various sources of bacteria in the watershed. However, much of the data used for the model was taken from literature sources due to a lack of actual data from the watershed. The limited data available from the watershed creates challenges in the determination of implementation strategies that will be the most successful in decreasing the amount of bacteria entering surface water in the Leon Watershed.

During the development of the TMDL for the Leon Watershed, livestock and waste application fields were implicated as being significant sources of bacterial loading to the Leon River. Through the finalization of the TMDL and the initiation of the implementation stage, an increased knowledge of the actual levels of bacteria in livestock waste and best management practices that reduce the runoff of bacteria from waste application fields will assist in decreasing movement of bacteria to surface water.

Actual data taken from sources in the Leon Watershed would assist in the development and implementation of the TMDL. Monitoring of bacterial sources listed in the TMDL will be beneficial in determining the sources of greatest bacterial concentrations and will assist in determining the risks associated with a variety of management practices on livestock operations.

Decreasing nutrient and bacteria loads in a watershed is dependent on the education of residents in the watershed. Providing resources to educate residents as to the best management practices that can be used to reduce movement of bacteria to surface waters is essential to the development of a successful TMDL implementation phase. Collection of data in the watershed will provide and increase understanding of bacteria loads in the watershed and will provide knowledge for areas that should be targeted to reduce the risks of bacteria from moving off the land and into surface waters.

A6. PROJECT/TASK DESCRIPTION

The overall objective of the project is to collect watershed specific data in an effort to quantify the major sources of *E. coli* bacteria on dairy operations. Information and data collected during the monitoring phase will be used in the development of an educational program focusing on best management practices (BMPs) to reduce the movement of *E. coli* bacteria and nutrients to surface waters. The educational program will equip dairy producers with the knowledge and understanding needed to reduce the possibility that their operations will be a source of bacteria and nutrients to the Leon River watershed (Fig.1). The monitoring and the educational programs will be designed to coordinate with the development of a TMDL implementation plan or a watershed plan, and will provide information and assistance for future watershed planning needs.

The tasks of the project are to:

1. Development of a QAPP.
2. Evaluate *E. coli* concentrations in manure and wastewater from dairy operations.
3. Evaluate *E. coli* and nutrient concentrations in surface water upstream and downstream of dairy waste application fields.
4. Evaluation of BMPs on loads of *E. coli* and nutrients in runoff from dairy fields.
5. Education of dairy producers and the community as to the presence of *E. coli* bacteria and nutrients in manure and wastewater, and BMPs to decrease *E. coli* bacteria and nutrients in runoff from dairy fields.

The objective of the first task is to develop and deliver to TSSWCB the QAPP for EPA approval.

The objective of the second task will be to determine the concentration of *E. coli* bacteria and nutrients present in dairy manure and wastewater throughout first year of the project. Samples of solids and wastewater from dairy lagoons on 4 operations will be collected on a monthly basis. This information will be supplemented with the collection of manure and wastewater samples during field application, up to six times per year, over a 3 year period. The samples will be analyzed to determine the forms and sources of manure containing the greatest bacteria and nutrient concentrations, thus posing the greatest opportunity for improvement in management strategies. (1 month to 36 months)

The goal of the third task will be to assess the concentrations of *E. coli* bacteria and nutrients in surface waters that are located adjacent to waste application fields. Currently, buffer strips are used to reduce the movement of manure and wastewater to surface waters. Monitoring surface waters at upstream and downstream sites will assist in determining if the bacterial and nutrient concentrations are being increased as a result of the waste application field. In particular, application and storm events, up to 10 combined events per year, will be monitored as these events pose the greatest opportunities for movement of bacteria and nutrients to surface water. Grab samples will be collected up (541136°E, 3528813°N and 541664°E, 352947°N) and down stream from the LMU. The LMUs are very close together, therefore there will be only two upstream and downstream sample locations. *E. coli* and nutrients will be determined on upstream and downstream samples collected quarterly from the two selected sites for duration of the project. (6 to 47 months)

The goal of the fourth task is to determine the effectiveness of different BMPs on reducing *E. coli* bacteria and nutrients in runoff from dairy fields.A total of two sites will be selected in Comanche County in the Leon River watershed (Fig. 1) for the evaluation of BMPs. Within each of the two sites, buffer strips, managed and unmanaged, will be established to give a total of 9 plots. The two fields will consist of two manured (wastewater and dry manure) fields (corn, hay, and pasture) and one inorganic fertilized filter strip. The manured fields will have buffer strips, one managed and one unmanaged. Each field will be set up for edge of field monitoring using ISCO samplers. An ISCO will be placed prior to the buffer at the edge of the field and after each buffer, grass buffer strips and riparian buffer strips, at the edge of that land management unit (LMU). Runoff from storm events will be collected by the ISCOs. *Escherichia coli* numbers and nutrients (NO_3-N, P, K, Ca, Mg, Na, and S) will be analyzed from each runoff sample and dissolved oxygen will be analyzed on the grab samples. Bacterial source will be analyzed on selected downstream samples. (12 month to 47 months)

Rainfall simulations will also be conducted on the field and the buffer strips in Task 4. Rainfall simulations will be conducted to measure simulated runoff *E. coli* and nutrient levels from field sites. A Phosphorus Index (PI) will be determined for each of the fields and specific locations within each plot for the simulations will be selected that best represents the PI characteristics and properties upon which the characterization was based. The rainfall simulations will be conducted using a Tlaloc 3000 rainfall simulator built by Joern's Inc. All rainfall simulation procedures will be conducted in accordance with the Sera-17 National P Project guidelines for rainfall simulations. A total of 4 rainfall simulation replications will be conducted at each of the two fields and filter strips per year. Runoff samples (100 mL) will be collected during each simulation at seven intervals (5, 10, 15, 20, 25, and 30 minutes plus a composite) after runoff is initiated. Each of the timed interval samples will be analyzed for pH and EC in the field, then acidified to pH 2 in the field with HCl. Three composite samples will be collected, one sample for nutrient (P, K, Ca, Mg, Na, and S) analyses except NO_3-N that will be acidified, one for NO_3-N that will not be acidified, and one for *E. coli* that will not be acidified. Cumulative runoff volume will be recorded at one minute increments throughout the 30 minute duration. Water samples will be analyzed for *E. coli* and nutrients as specified above. Soil samples (0-5, 5-15, and 0-15 cm (0-2, 2-6, and 0-6 inches)) will also be collected for each rainfall simulation from each of the four plots. Soil samples will be analyzed for pH; EC; Mehlich-3 P, K, Ca, Mg, Na, and S; and Cd reduction NO_3-N. (20 to 47 months)

The goal of task 5 is the education of dairy producers and the community as to the presence of *E. coli* bacteria and nutrients in manure and wastewater, and BMPs to decrease *E. coli* bacteria and nutrients in runoff from dairy fields. Results of the result demonstrations will be delivered to producers, TCEQ, TSSWCB, and other Texas clientele through Dairy Outreach Program Area meetings and multi-county meetings in the dairy areas of Texas. (12 to 47 months)

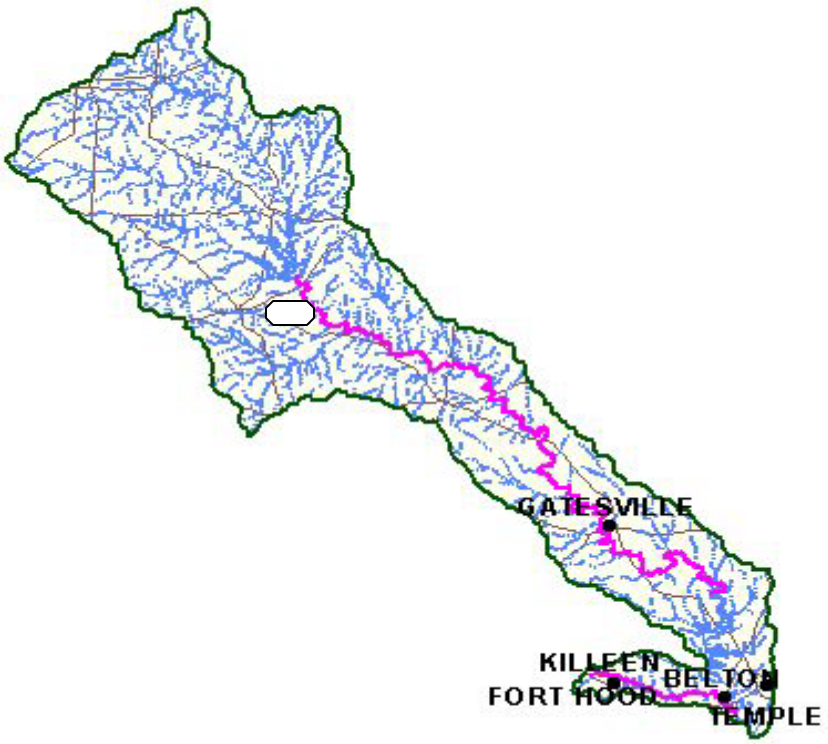


Figure 1. Study area in the Leon River waterbody segment (1221, south from Lake Proctor to Lake Belton)

A7. QUALITY OBJECTIVES AND CRITERIA

The objective of this section is to ensure that data collected meets the data quality objectives (DQOs) of the project. The measurement performance specifications to support the project objectives for a minimum data set are specified in Table A7-1 and Table A7-2 and in the text following.

The objectives of the sample collection portion of this project are as follows:

1. Evaluation of *E. coli* and nutrient concentrations in manure and wastewater from dairy operations.
2. Evaluation of *E. coli* and nutrient concentrations in surface water upstream and downstream of dairy waste application fields.
3. Evaluation of BMPs on *E. coli* and nutrient loading in runoff from dairy fields.

Achievement of these objectives will support decisions on how to target best management practices to reduce *E. coli* and nutrient levels in the Leon River Watershed.

Ambient Water Reporting Limits (AWRL)

The AWRLs specified in Table A7-1 and A7-2 are the program-defined reporting specifications for each analyte and yield data acceptable for routine water quality monitoring. The reporting limit is the lowest concentration at which the laboratory will report quantitative data within a specified recovery range. The laboratory will meet two requirements in order to report meaningful results to the TSSWCB:

- The laboratory's reporting limit for each analyte will be at or below the AWRL.
- The laboratory will demonstrate and document on an ongoing basis the laboratory's ability to quantify at its limit of quantitation (LOQ).

Acceptance criteria are defined in Section B5.

Table A7-1. Data Quality Objectives for Bacterial Measurement Data

Parameter	Units	Method Type	Method	Method Description	Storet	LOQ	Precision of Laboratory Duplicates*	Bias	Precision of Field Duplicates	Percent Complete
Lab Parameters										
E. coli in water	CFU/ 100 mL	Membrane filter culture on modified mTEC agar	Modified EPA Method 1103.1	Membrane Filter	31648	1	3.27* ΣRlog/n	NA	NA	90
E. coli ribotype	NA	DNA/ image matching	EP AREC SOP	Ribotyping	NA	NA	90% identical	90% correct	75% agreement	90#
E. coli PFGE pattern	NA	DNA/ image matching	CDC SOP	PFGE	NA	NA	90% identical	90% correct	75% agreement	90#
E. coli ERIC-PCR profile	NA	DNA/ image matching	EP AREC SOP	ERIC-PCR	NA	NA	90% identical	90% correct	75% agreement	90#
E. coli antibiotic resistance profile	NA	Culture-based	NCCLS Standard	ARA	NA	NA	90% identical	90% correct	75% agreement	90#

#The objective is for 90% of the data to be collected. An additional objective for BST completeness is that sources for 70% of host-specific isolates can be identified.
* Bias and laboratory method precision will be determined using isolates from known-source samples in a blind procedure, as discussed in Section B5.

Table A7-2. Estimated Accuracy and Precision Limits of Measured Nutrient Parameters

NA = Not applicable; mg/L = milligrams per liter; mL = milliliters; mg/kg = milligrams per kilogram; dS/m = decisiemens per meter;

Parameter	Precision Limits[1] (RPD)	Bias	SWFTL[2] Code	LOQ[3]
Laboratory Parameters				
Soil				
pH	NA	±0.2	0015	0.2 pH units
Electrical Conductivity	NA	± 2% of range	0015	0.05 dS/m
Nitrate-Nitrogen	20%	80-120%	0089	1.0 mg/kg
Phosphorus	20%	80-120%	0079	1.0 mg/kg
Potassium	20%	80-120%	0079	5.0 mg/kg
Calcium	20%	80-120%	0079	10 mg/kg
Magnesium	20%	80-120%	0079	5.0 mg/kg
Sodium	20%	80-120%	0079	10.0 mg/kg
Sulfate-Sulfur	20%	80-120%	0079	5.0 mg/kg
Runoff				
pH	NA	± 0.2 units	0041	0.2 pH units
Electrical Conductivity	NA	± 2% of range	0040	0.05dS/m
Nitrate-Nitrogen	20%	80-120%	0038	0.1 mg/L
Phosphorus	20%	80-120%	0037	0.2 mg/L
Potassium	20%	80-120%	0037	5.0 mg/L
Calcium	20%	80-120%	0037	10 mg/L
Magnesium	20%	80-120%	0037	5.0 mg/L
Sodium	20%	80-120%	0037	5.0 mg/L
Sulfate-Sulfur	20%	80-120%	0037	5.0 mg/L
Manure/Wastewater				
Nitrogen	20%	80-120%	0073	200.0 mg/kg
Phosphorus	20%	80-120%	0074	200.0 mg/kg
Potassium	20%	80-120%	0074	200.0 mg/kg
Calcium	20%	80-120%	0074	200.0 mg/kg
Magnesium	20%	80-120%	0074	200.0 mg/kg
Sodium	20%	80-120%	0074	200.0 mg/kg
Zinc	20%	80-120%	0074	3.0 mg/kg
Iron	20%	80-120%	0074	3.0 mg/kg
Copper	20%	80-120%	0074	3.0 mg/kg
Manganese	20%	80-120%	0074	3.0 mg/kg
Moisture	NA	± 2%	0080	1 %
pH	NA	± 0.2 units	0071	0.2 pH units
Electrical Conductivity	NA	± 2% of range	0072	0.05dS/m

[1] RPD = relative percent deviation
[2] SWFTL = Soil, Water and Forage Testing Laboratory, SOP code
[3] Estimated MRL for AgriLife Extension laboratory parameters as of February 22, 2005. LOQs for laboratory parameters are reevaluated about once every six months.

Precision

Laboratory precision is assessed by comparing replicate analyses of laboratory control standards. Precision results are plotted on quality control charts, which are based on historical data and used during evaluation of analytical performance. Program-defined measurement performance specifications for laboratory control standard/laboratory control standard duplicate pairs are defined in Table A7-1 and Table A7-2.

Precision is strictly defined as the degree of mutual agreement among independent measurements as the result of repeated application of the same process under similar conditions. It is assessed by repeated analyses of a sample. For quantitative microbiological analyses, the method to be used for calculating precision is outlined in Standard Methods for the Examination of Water and Wastewater, 20th Edition, section 9020 B.8.b.

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

The RPDbacteria 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.

The precision of the ERIC-PCR, ribotyping, PFGE, and ARA procedures can be measured as the percent of *E. coli* isolates that, when typed multiple times, produce the same ultimate source result in terms of the source identified.

More important, perhaps, is the precision of the overall result, including culturing, typing, library matching and interpretation. This can be measured through the use of field duplicates, by collecting duplicate water samples into two bottles at the time of collection, and processing them in an identical manner. However, because only a small portion of the total number of bacteria in a sample is typed, and the bacteria in a sample are expected to originate from various sources, the results for a given pair of duplicate samples are not expected to agree. However, by completely duplicating all the samples at a given site, the results of all samples combined should be in reasonable agreement with regard to source contribution percentages if sufficient samples are collected.

Bias

Bias is a statistical measurement of correctness and includes components of systemic 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 standards and limit of quantitation (LOQ) check standards prepared with certified reference materials and by calculating percent recovery. Results are plotted on quality control charts, which are calculated based on historical data and used during evaluation of analytical performance. Program-defined measurement performance specifications for bias (laboratory control standards) are specified in Table A7-1 and Table A7-2.

In BST, accuracy is best quantified through ribotyping/ARA/PFGE/ERIC-PCR of *E. coli* isolated from known sources as "double-blind" samples selected by a third party. Performance limits are specified in Table A7-1.

An additional element of bias is the absence of contamination. This is determined through the analysis of blank samples of sterile water processed in a manner identical to the sample.

Representativeness

Site selection, the appropriate sampling regime, the sampling of all pertinent media according to standard scientific SOPs, and use of only approved analytical methods will assure that the measurement data represents the conditions at the site.

Comparability

Confidence in the comparability of fixed/routine data sets for this project and for water quality assessments is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP.

Completeness

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

An additional element of completeness is involved with BST. The sources of *E. coli* isolates which do not match those from a library of known sources cannot be identified. In all BST studies, a source cannot be identified with acceptable confidence for a portion of the *E. coli* isolates. This is a function of 1) the size of the library relative to the true diversity of *E. coli* in the watershed, 2) the ability of the method to distinguish sources with acceptable confidence, and 3) the abundance of *E. coli* strains that colonize multiple sources, and thus cannot be used to uniquely identify a source. It will be a general goal of this project to identify the sources of 70% of the *E. coli* strains isolated from water.

A8. SPECIAL TRAINING/CERTIFICATION

No special certifications are required for sample collection or analyses. AgriLife Extension will ensure new field personnel (Tarleton State University or Texas A&M University students) will receive training in proper sampling and field analysis. Before actual sampling or field analysis occurs, they will demonstrate their ability to properly perform field sampling and analysis procedures.

A9. DOCUMENTS AND RECORDS

The documents and records that describe, specify, report, or certify activities are listed in Table A9-1.

Individual laboratory notebooks, which contain printouts of laboratory data and hand written observations and data, are kept by individual analysts at AgriLife Extension and AgriLife Research or the AgriLife Extension project manager for at least five years. When lab notebooks are filled, they are stored for at least five years by the laboratory manager in hardcopy form. AgriLife Extension / AgriLife Research laboratories keep their electronic data on personal computers for the duration of the project and then in hardcopy files for 5 years after the project. The original field data sheet is filed in a three-ring binder, according to site location and project, and stored for at least five years. COCs and attached documents are stored in numerical order in three-ring binders in the AgriLife Extension Data Manager's office for at least five years. In addition, the AgriLife Extension project manager will archive electronic forms of all project data for at least five years on personal computers and AgriLife Extension / AgriLife Research fire-resistant cabinets. A blank CAR form is presented in Appendix E, a blank COC is presented in Appendix C, and a blank field data reporting form is presented in Appendix F.

Any items or areas identified as potential problems and any variations or supplements to QAPP procedures noted in the laboratory quality assurance/quality control report will be made known to pertinent project personnel and included in an update or amendment to the QAPP.

Quarterly progress reports will note activities conducted in connection with the soil and water analyses, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary. CARs will be maintained in an accessible location for reference at AgriLife Extension. 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.

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

Laboratory Data Reports

Data reports from the AgriLife Extension and EP AREC laboratories will report the test results clearly and accurately. The test report will include the information necessary for the interpretation and validation of data and will include the following:

- name and address of the laboratory
- name and address of the client
- a clear identification of the sample(s) analyzed
- identification of samples that did not meet QA requirements and why (e.g., holding times exceeded)
- date of sample receipt
- sample results
- clearly identified subcontract laboratory results (as applicable)

- a name and title of person accepting responsibility for the report
- project-specific quality control results to include LCS sample results (% recovery), LCS duplicate results (%RPD), equipment, trip, and field blank results (as applicable), and LOQ confirmation (% recovery)
- narrative information on QC failures or deviations from requirements that may affect the quality of results.

In addition, a lab data report from the EP AREC laboratory, with sample results and QC results, will be submitted to AgriLife Extension for inclusion with project data submittals.

Table A9-1. Project Documents and Records

Document/Record	Location	Retention (yrs)	Format
QAPPs, amendments and appendices	AgriLife Extension – College Station	5 years	Paper/Electronic
Field SOPs	AgriLife Extension – College Station, AgriLife Research - Stephenville	5 years	Paper/Electronic
Laboratory QA Manuals	AgriLife Extension – College Station, AgriLife Research	5 years	Paper/Electronic
Laboratory SOPs	AgriLife Extension – College Station, AgriLife Research	5 years	Paper/Electronic
QAPP distribution documentation	AgriLife Extension – College Station, AgriLife Research	5 years	Paper
Field staff training records	AgriLife Research – Stephenville, AgriLife Extension – College Station	5 years	Paper
Field equipment calibration/maintenance logs	AgriLife Research – Stephenville, AgriLife Extension – College Station	5 years	Paper
Field notebooks or data sheets	AgriLife Research – Stephenville, AgriLife Extension – College Station	5 years	Paper/Electronic
Chain of custody records	AgriLife Extension – College Station, AgriLife Research	5 years	Paper
Laboratory calibration records	AgriLife Extension – College Station, AgriLife Research	5 years	Paper/Electronic
Laboratory instrument printouts	AgriLife Extension – College Station, AgriLife Research	5 years	Paper/Electronic
Laboratory data reports/results	AgriLife Extension – College Station, AgriLife Research	5 years	Paper/Electronic
Laboratory equipment maintenance logs	AgriLife Extension – College Station, AgriLife Research	5 years	Paper
Corrective Action Documentation	AgriLife Extension – College Station, AgriLife Research	5 years	Paper

*AgriLife Research Lab refers to Texas AgriLife Research- El Paso, El Paso, TX 79927

Special Reporting Formats

Ambient *E. coli* concentration data will be reported in accordance with standard data formats. RiboPrinting and ERIC-PCR data will be maintained as electronic and hard copy image files. Library matching statistics will be reported in the final report.

Electronic Data

Project data will be submitted electronically to AgriLife Extension project manager in Excel files as e-mail attachments (sfeagley@ag.tamu.edu) besides a hardcopy. The electronic files will be stored at AgriLife Research-Stephenville and AgriLife Extension-College Station (nutrient soil and water analyses) and files will be maintained on the Stephenville S (shared) drive. In addition, hardcopies will be stored at AgriLife Research-Stephenville and AgriLife Extension-College Station (nutrient soil and water analyses). Individuals in A3 will be sent the most current copy of the QAPP by the AgriLife Extension project manager.

Backup/Disaster Recovery

The S drive and the network server are backed up daily to a tape drive at Stephenville. The nutrient data is backed up daily using a Buffalo 500 hard drive on all computers used by Sam Feagley's group. The bacteria and source tracking data is backed up using protocol accepted by TCEQ for NELAC accredited laboratories. In the event of a catastrophic systems failure, the tapes can be used to restore the data. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

Amendments to the QAPP

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. Requests for amendments are directed from the AgriLife Extension Project Manager to the TSSWCB Project Manager in writing. They are effective immediately upon approval by the TSSWCB Project Manager and the EPA Project Officer. They will be distributed by the AgriLife Extension Project Manager and incorporated into the QAPP by way of attachment and distributed to personnel on the distribution list.

Expedited Changes

Expedited changes to the QAPP should be approved before implementation to reflect changes in project organization, tasks, schedules, objectives, and methods, address deficiencies and non-conformance, improve operational efficiency and accommodate unique or unanticipated circumstances. Requests for expedited changes are directed from the contractor AgriLife Extension Project Manager to the TSSWCB Project Manager in writing. They are effective immediately upon approval by the TSSWCB Project Manager and QAO.

Expedited changes to the QAPP and the reasons for the changes shall be documented, and revised pages shall be initialed by the AgriLife Extension and TSSWCB Project Managers and QAO, and the EPA Project Officer (if applicable), and then distributed to all persons on the QAPP distribution list by the AgriLife Extension Project Manager. Expedited changes shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process or within 120 days of the initial approval in cases of significant changes.

B1. SAMPLING PROCESS DESIGN

The main goal of this project is to evaluate the concentration of *E. coli* and nutrients in various dairy manure and wastewater streams and to evaluate BMPs for reducing the movement of *E. coli* and nutrients into surface water.

In order to obtain temporally representative results, including wet and dry conditions and seasonal variation, the sampling from dairy operations and ambient water sampling will occur on a routine schedule (once per month on the closest working day to the first of each month) over the course of 1 year, and capture various weather events at their natural frequency, as they occur. Samples will not be collected under dangerous conditions, but will be collected as soon as possible after the conditions have improved and the time and date will always be recorded. For instance, samples will not be collected during a thunderstorm, but after the storm and after runoff or high water (in stream) events are completed. Analyses to be completed on these samples will be *E. coli* and nutrients.

All samples will be replicated by virtue of trial/demonstration designs in which at least 2 replications will be used. This approach is necessary for accurate statistical analyses. Specific water, soil, and manure/wastewater sampling process designs are outlined in Appendix B.

A second goal of this project is to determine the source of *E. coli* isolates and to expand the library for *E. coli* bacteria. Confirmed *E. coli* bacterial colonies will be screened using a repetitive sequence polymerase chain reaction (ERIC-PCR) method. ERIC-PCR is a genetic fingerprinting method used for BST and will be used to identify unique *E. coli* isolates from each sample and eliminate further analysis of identical isolates (clones). *E. coli* will be isolated from each manure source, identified, and added to the Texas BST known-source library. At least one *E. coli* isolate from each sample will be included in the library. *E. coli* isolates will be obtained from edge of field samples collected from each of the 10 plots, along with upstream and downstream samples, four times a year (approximately every three months). These isolates will be compared against the known-source library for source identification.

A third goal of this project is to determine the variation in nutrient content in the soil and in runoff due to BMPs. The runoff analyses will be conducted after each runoff event and for each of the rainfall simulations as described in Section A6. Soil samples will be collected from each field in years three and four in September. Additional soil samples will be collected from each of the four rainfall simulations per plot per year. Rainfall simulations will be conducted in the spring of each year. Soil sample analyses will include pH; EC; Mehlich-3 P, K, Ca, Mg, Na, and S; and Cd reduction for NO_3-N.

B2. SAMPLING METHODS

Field sampling personnel will wear clean, disposable, powder-free gloves while collecting all samples for *E. coli* analyses, but not for soil or water nutrient analyses.

Field Sampling Procedures

Field sampling will be conducted according to the sample handling procedures described in Section B2 and 3 and in Appendix B.

Sample volume, container types, minimum sample volume, preservation requirements, and holding time requirements are presented in Table B2 and Table B2-1.

Water Samples

Water samples will be collected directly from the stream (approximately one foot below the surface) into sterile wide-mouthed polypropylene bottles supplied by the culturing laboratory in Stephenville. Care will be exercised to avoid the surface microlayer of water, which may be enriched in bacteria and not be representative of the water column. In cases where, for safety reasons, it is inadvisable to enter the stream bed, staff will use a clean plastic bucket and rope to collect the samples from the stream, and pour the water into the sample bottles. If a bucket is used, care will be taken to avoid contaminating the sample. 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 and the sampler's gloved hands. Samples are collected from subsequent buckets of water.

Upon collection, all water samples will be transported in an iced container to the laboratory for analysis.

Dairy manure and wastewater samples will be collected from four representative dairy operations as described in Section A6 in the Leon Watershed. The sample will be collected using a randomized sampling technique. Fifteen to 20 subsamples per dairy will be collected. After all subsamples are collected, the manure/wastewater will be thoroughly mixed and a sample for analyses collected from the mixture.

Runoff water collection will be done according to National P Benchmark Soils Project from portable 1.5 x 2.0m frames. One rainfall simulation will be conducted on each of 4 plots at each of the 4 locations, providing four replications for statistical comparison. Runoff samples (~125 mL) will be collected during each simulation at 6 intervals (5, 10, 15, 20, 25, and 30 minutes) after runoff is initiated and a composite (1000 mL for water and sediment (selected) analyses and 125 mL for NO_3-N). Runoff weight will be recorded every minute after runoff is initiated, and total runoff weight will also be recorded. Water samples that will be analyzed for *E. coli* and nutrients, except the NO_3-N sample, will have pH and EC analyzed and recorded in the field. All samples except the NO_3-N will be acidified to pH 2 with nitric acid following these analyses.

Water samples will be stored in an ice chest at approximately 4°C and transported to the research lab as soon as possible. Upon arrival to the research lab, *E. coli* samples will be analyzed immediately and samples to be analyzed for nutrients will be filtered. Samples for nutrient analyses will be stored in a refrigerator until analyses are completed in the AgriLife Extension SWFTL.

Table B2. Sample Procedures and Handling Methods for *E. coli* samples.

Parameter	Matrix	Container	Preservation	Temperature	Sample Volume	Holding Time
E. coli	water	Sterile Whirl-pak® bags	none	4°C	>200 ml	24 hours
E. coli	Manure /waste water	Sterile Whirl-pak® bags	none	4°C	>10 g	24 hours

6 hours to deliver to laboratory. The laboratory has an additional 2 hours to get the sample filtered and culturing on growth media.

Table B2-1. Sample Procedures and Handling Methods for Samples Collected for Nutrient Analyses.

Parameter	SWFTL	Container	Preservation	Temperature	Holding Time
Soil Parameters					
pH	0015	Sample Bag	Air Drying	25°C	NA
Electrical Conductivity	0015	Sample Bag	Air Drying	25°C	NA
Nitrate-Nitrogen	0089	Sample Bag	Air Drying	25°C	NA
Phosphorus	0079	Sample Bag	Air Drying	25°C	NA
Potassium	0079	Sample Bag	Air Drying	25°C	NA
Calcium	0079	Sample Bag	Air Drying	25°C	NA
Magnesium	0079	Sample Bag	Air Drying	25°C	NA
Sodium	0079	Sample Bag	Air Drying	25°C	NA
Sulfate-Sulfur	0079	Sample Bag	Air Drying	25°C	NA
Runoff Parameters					
pH	0041	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Electrical Conductivity	0040	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Nitrate-Nitrogen	0038	HDPE	None	4°C	28 days
Phosphorus	0037	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Potassium	0037	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Calcium	0037	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Magnesium	0037	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Sodium	0037	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Sulfate-Sulfur	0037	HDPE	Acid. HNO_3, pH 2	4°C	28 days
Manure/Wastewater					
Nitrogen	0073	Zip-lock bag	Air Drying	25°C	NA
Phosphorus	0074	Zip-lock bag	Air Drying	25°C	NA
Potassium	0074	Zip-lock bag	Air Drying	25°C	NA
Calcium	0074	Zip-lock bag	Air Drying	25°C	NA
Magnesium	0074	Zip-lock bag	Air Drying	25°C	NA
Sodium	0074	Zip-lock bag	Air Drying	25°C	NA
Zinc	0074	Zip-lock bag	Air Drying	25°C	NA
Iron	0074	Zip-lock bag	Air Drying	25°C	NA
Copper	0074	Zip-lock bag	Air Drying	25°C	NA
Manganese	0074	Zip-lock bag	Air Drying	25°C	NA
Moisture	0080	Zip-lock bag	Air Drying	25°C	NA
pH	0071	Zip-lock bag	Air Drying	25°C	NA
Electrical Conductivity	0072	Zip-lock bag	Air Drying	25°C	NA

SWFTL = Soil, Water and Forage Testing Laboratory Standard Operating Procedures (SOPs)
HDPE = High Density Polyethylene bottles
HNO_3 = concentrated nitric acid
°C = degrees centigrade
NA = not applicable, indefinite holding time after air drying

Sample Containers

E. coli water samples will be collected using sterilized Whirl-pak® bags. HDPE bottles will be used for water samples collected for nutrient analyses only. Sterile plastic Whirl-pak® bags will be used to collect manure samples. Sterilized bags will not be cleaned and reused.

Contamination resulting from improper washing and sterilization procedures will be determined by evaluating a blank for each batch of 20 samples. Sterilized buffer is poured into the BLANK Whirl-pak® bags and it is treated just like the samples being analyzed that day. If any measured concentration is greater than the AWRL, corrective actions will be initiated. Sources of contamination are investigated and remediated, if found. Corrective action documentation is maintained for BLANK failures. Corrective actions include reanalyzing to confirm method blank contamination, investigating the source of the contamination, identifying all samples possibly affected by the contamination, and conferring with the AgriLife Extension PM to determine if the data are acceptable.

Processes to Prevent Contamination

Samples will be collected directly into sample containers, when possible, to avoid contamination.

Documentation of Field Sampling Activities

Field sampling activities are documented on field data sheets, which are included in Appendix F. The following will be recorded for all samples:

1. Site location
2. Sampling time
3. Sampling date
4. Sampling location
5. Sample collector's name/signature
6. Values for all measured field parameters (as appropriate)
7. Preservative added, if applicable
8. Detailed observational data (as appropriate), including:
    - water/manure/soil appearance
    - weather
9. Other observational data (as applicable), including:
    - activities in contributing fields that could impact samples (events impacting water quality, e.g., livestock watering upstream, etc.)
    - unusual odors
    - specific sample information (number of grabs, type, etc.)
    - missing parameters (i.e., when a scheduled parameter or group of parameters is not collected)

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:

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

Deficiencies, Non-conformance and Corrective Action Related to Sampling Requirements

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

Deficiencies are documented in logbooks, field data sheets, etc. by field or laboratory staff and are reported to the cognizant field or laboratory supervisor via a corrective action report (CAR). The supervisor notifies the AgriLife Extension Project Manager if the deficiency has the potential of being a nonconformance. The AgriLife Extension Project Manager will notify the TSSWCB QAO of the potential nonconformance within 48 business hours.

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

CARs associated with non-conformances will be included with quarterly progress reports. In addition, significant conditions (i.e., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported TSSWCB both verbally and in writing.

B3. SAMPLE HANDLING AND CUSTODY

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis.

A sample is in custody if it is in actual physical possession or in a secured area that is restricted to authorized personnel. The chain of custody (COC) form is used to document sample identification and handling during transfer from the field staff to the AgriLife Extension laboratories and then to the EP AREC laboratory. For grab samples, a field data sheet for each site is attached to the COC.

The COC form is used to document sample handling during transfer from the field to the laboratory and among subcontract laboratories. The following information concerning the sample is recorded on the COC form (See Appendix C). These are standard requirements for COC forms.

1. Date and time of collection
2. Site identification
3. Sample matrix
4. Number of containers, if applicable
5. Preservative, if applicable
6. Color code to indicate required analyses
7. Name of collector
8. Custody transfer signatures and dates and time of transfer

The sample collector will sign the COC and transport it with the sample to the appropriate 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.

Sample Labeling

Water and manure samples are labeled on the container with an indelible marker. Label information includes:

1. Site location
2. Date of sample collection (MM/DD/YYYY format)
3. Time of sampling (or bottle number for composited samples)

These two unique identifiers can be matched with data on Chain of Custody forms when submitting samples. All samples are submitted on a same day basis and given a unique sample number. This sample identification number, time, and site location serve to match the sample

with the data on the COC. All water samples are submitted to the laboratory on ice. Project samples do not require additional types of preservation prior to receipt by the laboratory. No samples for this project are field filtered.

Sample Handling

Each sample container is labeled in the field with the identification stated above. Water samples are preserved on ice in a cooler while they are being transported to the laboratory. The field staff member documents in a field data sheet, COC form, or sample bench sheet the station, date, time, location, and sample type. A sample identification number is assigned to water samples at the AgriLife Extension laboratory and is written on the sample container and on the COC. The sample number, location, date, changes in possession and other pertinent data are recorded in ink on the COC, which accompanies all sets of sample containers. The field staff member transfers possession of the samples to a laboratory staff member or alerts a laboratory staff member and leaves the sample containers, COCs and other paperwork in a secured area. The field staff member and the laboratory staff member both sign and date the COC. Copies of the COC form used on this project are included as Appendix C.

Following the 24-hour culture incubation and subsequent enumeration, one Petri dish per sample containing a membrane filter on modified mTEC medium with 1 – 100 (preferably 10 – 40) *E. coli* colonies is labeled appropriately, placed in a sealable bag, and transferred to an insulated DOT-approved shipping container with blue ice for cooling. The AgriLife Extension laboratory staff will then enclose the sample COC in the shipping container and send it via overnight courier to the EP AREC laboratory.

Manure/Wastewater Samples. Manure or wastewater samples will be bagged in sealable plastic bags or bottles and marked with sample identification on the outside of the bag or bottle using a waterproof marker. The sample identification will identify the dairy and the manure/wastewater source from which the sample was taken. Plastic bags or bottles containing manure/wastewater samples will be placed on ice and transported to AgriLife Research Laboratory, Stephenville, Texas.

Soil Samples. Soil samples will be shipped to the AgriLife Extension-SWFTL for analysis. Each soil sample will be placed in a soil sample bag, with sample identification marked on the outside of the sample bag. The label on the soil sample bag will contain the sample identification number, the dairy/site location, and the depth(s) from which the sample was taken. An Excel spreadsheet will be completed for each day and site of sampling and printed in duplicate. One copy of the soil sample information sheet (Appendix H) will accompany the composite samples to the AgriLife Extension-SWFTL and one copy will be included in the project file at AgriLife Extension.

Laboratory Analysis and Data Collection

A Test Group code is marked on the COC by the field staff to designate the type of analytes to be measured for each sample. Upon receipt of samples and COC, the laboratory staff member compares the time of collection and the shortest holding time for the required analyses against the time of receipt to ensure that sufficient time has been allowed to complete the analyses.

When analyses are complete, the laboratory staff checks again to see whether the samples were analyzed within the holding time. This can become an issue when quality control checks are not met and the analysis must be repeated. Laboratory staff consistently monitors the remaining time for analyses and work to ensure that samples are analyzed within holding time restraints. Aliquots of each sample are used by the laboratory staff in running the various analytical procedures. The sample number is marked on all containers to which aliquots are transferred. Aliquots are filtered, as necessary, and analyzed as per standard operating procedures. Data pertaining to analyte measurements are recorded in bound personal logbooks, which are specific to each procedure and analyst. According to the type of analysis, measurement data produced in the laboratory is either printed out from the automated analytical equipment, read from screens on equipment and copied to Excel spreadsheets that calculate concentrations. Determination of *E. coli* concentrations will be conducted by manually counting colony numbers. Whenever possible, printouts of data from analytical equipment and from Excel spreadsheets are placed into the bound notebooks. Measurement data are copied from the notebooks to the computer database. Physicochemical data are downloaded from the databases and transferred electronically to the SAS database.

B4. ANALYTICAL METHODS

The analytical methods, associated matrices, and performing laboratories are listed in Table A7-1 and Table A7-2 of Section A7. Procedures for laboratory analysis will be in accordance with the most recently published edition of *Standard Methods for the Examination of Water and Wastewater*, the latest version of the *TCEQ Surface Water Quality Monitoring Procedures Manual*, 40 CFR 136, or other reliable procedures. Exceptions to this include analyses and sample matrices for which no regulated methods exist, or where USEPA has not approved any method with adequate sensitivity. In this project, these methods include all of the analyses for manure/wastewater and soil but no methods have been approved by USEPA. The analytical methods chosen to provide soils data include methods outlined in the Soil Science Society of America Soil Methods Book. The analytical methods chosen to provide forage tissue data and manure nutrient values are those outlined by the A.O.A.C. (Official methods of analysis, 15th Ed. Association of Official Analytical Chemists. Washington D.C., 1990.) and listed in Table A7-1 and Table A7-2.

The EC and pH of runoff water from simulated rainfall events will be measured in the field. The remainder of the parameters listed in Table B2 and Table B2-1 will be analyzed by AgriLife Extension in the SWFTL and research lab as specified in preceding sections, College Station, Texas. A listing of analytical methods and equipment is provided in Table B4-1. SOPs have been established for all of the procedures undertaken by AgriLife Extension SWFTL staff that concerns soil, water and manure analyses, and copies of the SOPs are available upon request.

In the event of a failure in the analytical system, the Project Manager will be notified. The Laboratory Manager, Quality Assurance Officer, and Project Manager will then determine if the existing sample integrity is intact, if re-sampling should and/or can be done, or if the data should be omitted.

Library Sample *EC* Isolation and Purification

Fecal specimens or wastewater samples will be streaked (resuspended in buffer if necessary) onto modified mTEC medium, a selective and differential medium for *E. coli*, and incubated at 35∀0.5EC for two hours to resuscitate stressed bacteria, then incubated at 44.5∀0.2EC for approximately 20-24 hours. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate. The modified medium contains 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 other substrates such as MUG and UV fluorescence as used in other *E. coli* assays (e.g. IDEXX QuantiTray). *E. coli* colonies from the modified mTEC medium will be picked and streaked for purity on nutrient agar with MUG (NA-MUG) and glucuronidase activity and culture purity confirmed.

Water Samples *EC* Isolations and Purification

E. coli in water samples will be isolated and enumerated by the AgriLife Extension laboratory using modified mTEC agar, Modified EPA Method 1103.1 (Improved Enumeration Methods for

the Recreational Water Quality Indicators: Enterococci and Escherichia coli. EPA/821/R-97/004. March 2000.). The resulting plates will then be shipped via overnight courier to the EP AREC laboratory. Colonies will be confirmed as *E. coli* via the procedures described above for library samples.

E. coli Analysis

Confirmed *E. coli* bacterial colonies will be screened using a repetitive sequence polymerase chain reaction (ERIC-PCR) method. ERIC-PCR is a genetic fingerprinting method used for BST and will be used to identify unique *E. coli* isolates from each sample and eliminate further analysis of identical isolates (clones). At least one *E. coli* isolate from each manure and wastewater sample will be included in the library, even if it is identical to a previously isolated *E. coli*. Therefore, abundant/common strains will be sufficiently represented in the libraries. It is anticipated that approximately 3,000 *E. coli* colonies will be screened by ERIC-PCR. Cultures of selected isolates will be archived in tryptone soy broth (TSB) with 20% glycerol at -70 °C in cryovials and subcultures will be shipped to the other investigators for further analysis.

Following ERIC-PCR analysis, selected isolates will shipped via overnight courier to the EP AREC. These isolates will be ribotyped using the Qualicon automated RiboPrinter using the restriction enzyme Hind III. The isolates will be further characterized using pulsed-field gel electrophoresis (PFGE) at the EP AREC laboratory.

The analytical methods are listed in Table A7-1 of Section A7. No USEPA-approved methods exist for ERIC-PCR, ribotyping, PFGE, or ARA. The ARA method is a standard method of the National Committee for Clinical Laboratory Standards (NCCLS). The PFGE method is that of the federal CDC. These methods are provided in Appendices L, M, N, and O.

Copies of AgriLife Extension laboratory SOPs are retained by the AgriLife Extension. Copies of AgriLife Research SOPs are retained by AgriLife Research.

Standards Traceability

All standards used in the field and laboratories are traceable to certified reference materials. Standards preparation is fully documented and maintained electronically and in hard copies (AgriLife Research- Stephenville) imbedded in the data sets for which they served as standards. Each documentation includes information concerning the standard identification, starting materials, including concentration, amount used and lot number; date prepared, expiration date and preparer's name. The reagent bottle is labeled with the stock solution/dry chemical used in preparation of the reagent.

Table B4-1. Laboratory Analytical Methods

Parameter	SWFTL	Equipment Used
Soil Parameters		
pH	0015	pH meter
Electrical Conductivity	0015	Conductivity meter
Nitrate-Nitrogen	0089	Nitrate analyzer (Cd reduction)
Phosphorus	0079	ICP, Colorimetric (selected samples)
Potassium	0079	ICP
Calcium	0079	ICP
Magnesium	0079	ICP
Sodium	0079	ICP
Sulfate-Sulfur	0079	ICP
Runoff Parameters		
pH	0041	pH meter
Electrical Conductivity	0040	Conductivity meter
Nitrate-Nitrogen	0038	Nitrate analyzer (Cd reduction)
Phosphorus	0037	ICP
Potassium	0037	ICP
Calcium	0037	ICP
Magnesium	0037	ICP
Sodium	0037	ICP
Sulfate-Sulfur	0037	ICP
Manure/Wastewater		
Nitrogen	0073	Nitrate analyzer (Cd reduction)
Phosphorus	0074	ICP
Potassium	0074	ICP
Calcium	0074	ICP
Magnesium	0074	ICP
Sodium	0074	ICP
Zinc	0074	ICP
Iron	0074	ICP
Copper	0074	ICP
Manganese	0074	ICP
Moisture	0080	Metler Balance
pH	0071	pH meter
Electrical Conductivity	0072	Conductivity meter

SWFTL = Soil, Water and Forage Testing Laboratory Standard Operating Procedures (SOPs)

B5. QUALITY CONTROL

Table A7-1 and Table A7-2 list the required bias, precision, and completeness limits for the parameters of interest.

Bottle and Equipment Blanks

An equipment blank is a sample of reagent water poured into a sample bottle, or poured over or pumped through a sampling or analysis device. It is collected in the same type of container as the environmental sample, preserved in the same manner and analyzed for the same parameter. In addition to regularly collected bottle and equipment blanks, laboratory equipment blanks are prepared at the laboratory where collection materials are cleaned between uses. These blanks document that the materials provided by the laboratory are free of contamination. The QC check is performed with each new batch of equipment or bottles. The analysis of equipment blanks should yield values less than the MAL. When target analyte concentrations are very high, blank values must be less then 20% of the lowest value of the batch.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

The AgriLife Extension SWFTL and AgriLife Extension research laboratories will determine the precision of their analyses. Annual laboratory audits, sampling site audits, and quality assurance of field sampling methods will be conducted by AgriLife Extension QA officers. In addition to these annual audits, an independent laboratory and field audit will be conducted once during the course of this project by the TSSWCB project manager and QAO.

There will be no spiked sample analyses. The reason no spikes can be used is due to the different adsorptive capacities of different soil types for most of the elements being measured in this study. Therefore, adding elements to soils or runoff containing soil particles would always yield varying returns due to the chemical properties of soils.

The use of approved sampling and analytical methods will ensure that measured data accurately represent field conditions. Table A7-1 and Table A7-2 in Section A7 “Quality Objectives and Criteria” lists the appropriate bias for the parameters of interest. The completeness of the data will be affected by the reliability of the equipment, frequency of field and laboratory errors or accidents, and unexpected events; however, the general goal requires 90 percent data completion.

In the database, missing values will be left as blanks. Graphical screening of the data will be used to highlight questionable data points. Questionable data will be traced through the COC forms, CARs, and, as necessary, through research laboratory notebooks and field data sheets to ensure that data are properly entered. Changes will be made only if an error is found in transcription into database. Values determined to be below the laboratory method detection limit will be noted as such in the comment column of the database and used in statistical analyses as one-half the method detection limit (MDL), as recommended by Gilliom and Helsel (1968) and Ward et al. (1988). Values that are greater than the upper method detection limit will be diluted or re-extracted at a lower soil to extractant ratio and reanalyzed.

It is the responsibility of the project manager to verify that the data are representative. The chemistry data's precision, bias, and comparability generated in the AgriLife Extension laboratories or the AgriLife Extension SWFTL will be the responsibility of the laboratory director. The project manager has the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents at AgriLife Extension requiring corrective action will be documented through use of CARs (Appendix E).

Deficiencies, Non-conformances and Corrective Action Related to Quality Control

Deficiencies are documented in logbooks or field data sheets by field or laboratory staff and are reported to the cognizant field or laboratory supervisor via a corrective action report (CAR). The supervisor notifies the AgriLife Extension Project Manager if the deficiency has the potential of being a nonconformance. The AgriLife Extension Project Manager will notify the TSSWCB QAO of the potential nonconformance within 48 business hours.

The AgriLife Extension Project Manager, in consultation with TSSWCB QAO (and other affected individuals/ organizations), will determine if the deficiency constitutes a nonconformance. If it is determined a nonconformance does exist, the AgriLife Extension Project Manager in consultation with the TSSWCB QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented on the CAR. CARs associated with non-conformances will be included with quarterly progress reports. In addition, significant conditions (i.e., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported TSSWCB both verbally and in writing.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

EOF sampling equipment (ISCOs) is inspected and tested upon receipt and is assured appropriate for use by the AgriLife Extension specialists. Equipment records are kept on all field equipment and a supply of critical spare parts is maintained. If problems cannot be remediated on-site during the inspection, required adjustments or repairs will be made as soon as possible.

All laboratory tools, gauges, instrument, and equipment testing and maintenance requirements are contained within laboratory standard operating procedures. Testing and maintenance records are maintained and are available for inspection. Instruments requiring daily or in-use testing include, but are not limited to, water baths, ovens, autoclaves, incubators, refrigerators, and laboratory-pure water. Critical spare parts for essential equipment are maintained to prevent downtime. Maintenance records are available for inspection.

B7. INSTRUMENT CALIBRATION AND FREQUENCY

Detailed laboratory calibrations are contained within the standard operating procedures. AgriLife Extension and AgriLife Research standard operating procedures identify all tools, gauges, instruments, and other sampling, measuring, and test equipment used for data collection activities affecting quality that must be controlled and, at specified periods, calibrated to maintain bias within specified limits. Calibration records are maintained, are traceable to the instrument, and are available for inspection by the TSSWCB. Deficiencies will be resolved by the technicians responsible for the equipment or by company engineers and evidence of these calibrations will be maintained by the technicians and will be available for inspection at any time.

Specific instruments requiring calibration are listed in Table B4. All instruments will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendations in Standard Methods for the Examination of Water and Wastewater, 20th Edition, Section 9020 (APHA, 1998). Documentation of instrument calibrations is maintained in each laboratory. Calibration records are available to the TSSWCB for review.

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 (ACS) or reagent grade quality, or of the best attainable grade. All certified standards will be maintained traceable with certificates on file in the laboratory. Dilutions from all standards will be recorded in the standards log book and given unique identification numbers. The date, analyst initials, stock sources with lot number and manufacturer, and how dilutions were prepared will also be recorded in the standards log book.

Table B7. Instrument Calibration Requirements

Equipment	Relevant Calibration Requirement
Thermometers	SM 9020B 3.a
Balances	SM 9020B 3.b
pH meter	SM 9020B 3.c
Qualicon Riboprinter	manufacturer’s instructions using standard bacterial strain
gel electrophoresis apparatus	manufacturer’s instructions using standard bacterial strain

Any laboratory-specific differences from these requirements are noted below:

AgriLife Extension and EP AREC – balances are calibrated using approved weights by laboratory personnel and are calibrated yearly by industry representatives.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All new batches of field and laboratory supplies and consumables received by the AgriLife Extension laboratory are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Chemicals, reagents, and standards are logged into an inventory database that documents grade, lot number, manufacturer, dates received, opened, and emptied. All reagents shall meet ACS grade or equivalent where required. Acceptance criteria are detailed in organization's standard operating procedures. Supplies for microbiological analysis are received pre-sterilized, used as received, and not re-used. The laboratory standard operating procedures provide additional details on acceptance requirements for laboratory supplies and consumables.

B9. NON-DIRECT MEASUREMENTS

Literature files collected by the project manager and other members of the project team will be consulted for non-direct data sources. Additional sources will be accessed via the Texas A&M libraries and its electronic facilities at College Station. Resources will include desktop computers utilized to access the A&M libraries via the Internet as well as file cabinets and shelves in each individual's office. Using these resources, the project manager will assist with project members in determining validity and operating conditions proposed in the literature and measured in the field.

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B10. DATA MANAGEMENT

Data Management Process

Section B3 contains a detailed discussion of how samples are handled from collection through delivery to the laboratories. Included within that discussion is a description of how station information is taken and recorded on COC and other data forms. This section continues with the manner in which data are handled by AgriLife Extension and AgriLife Research until they are submitted to TSSWCB. In addition, this section outlines the data management associated with samples submitted to the AgriLife Research Stephenville Laboratory, AgriLife Extension SWFTL, and EP AREC laboratory.

AGRILIFE EXTENSION Water, Manure, and Wastewater Data Entry

As described in Section B3, generated data entered on the COCs and in laboratory logbooks are passed on to project managers. Afterwards, a data analyst reviews the COCs for correctness, abnormalities, and problems. Dairy/Site names (or numbers), plot descriptions, appropriateness of data values, completeness of data, dates and times, bottle numbers, start and end times of composited samples, comments and all other data on the sheets are reviewed. Any questions or abnormalities are investigated, relying largely on field and laboratory data sheets, general maintenance sheets, field technicians, laboratory notebooks, sampler printouts, compositing program printouts, and laboratory personnel. Any errors are crossed out and the correct data are added. Corrective action reports are completed, as appropriate.

Field Collection and Management of Simulated Rainfall Samples

Once sites are selected, rainfall simulations will be scheduled with individual land owners. The plot frames will be installed and the plot area pre-wet the day before the actual simulations. Water will be obtained from the closest municipality from a water hydrant if at all possible to decrease the amount of time required to fill the 1,100 gallon tank. This water is not treated for the pre-wetting and is passed through the water treatment columns for rainfall simulations. Rainfall simulators will be calibrated daily for flowrate of 7.5 cm/hr. One rainfall simulation will be conducted on each of four plots at each site. Runoff water will be collected during each of the simulations and soil samples following the simulation. These samples will be collected in specified containers, stored according to protocol (Table B2 and Table B2-1), and analyzed according to specified parameters (Table B4-1). It will take approximately two days at each site. A field data sheet and COC form will be completed at each site as shown in Appendix C.

The pH and EC will be measured on the water samples in the field prior to acidification and placing the samples in the ice chest. Each soil, water, and if available, manure/wastewater sample will be given a unique sample number and the sample container labeled by two different methods to assure sample identification. Sample ID numbers are recorded on the COC forms. Samples for nutrient analyses will be transported to the laboratory as soon as the field sampling crew returns. This may be as soon as one day or as long as one week. Samples will be stored according to protocol during transportation. When samples enter the AgriLife Extension

SWFTL, a unique lab number will be assigned. This number will be carried through the lab and reunited with the field number when the report is generated.

Sample containers being processed are typically placed in order of sample number, so the order of the sample containers matches the order of the field data and the COC sample ID numbers, reducing transcription errors. Sample number, comments, and other pertinent data are copied from the field data sheets to the COC. The COC and accompanying sample containers are submitted to the lab, with relinquishing and receiving personnel both signing and dating the COC.

EP AREC and Soil & Aquatic Microbiology Laboratory Data

Data collection will begin upon receipt of fecal specimens or modified mTEC plates with presumptive *E. coli* isolates. Unique identification numbers will be developed for each sample and for each isolate and will be recorded in handwritten notebooks, the chain of custody log and electronic database. The molecular analysis of isolates will be recorded both as a digital image and as a printed image in the research personnel's notebook. All electronic data and records will be backed up weekly on separate storage media. The laboratory supervisor will review the results weekly to examine the quality of data being recorded. The laboratory supervisor or his designee will provide draft electronic copies of the data on at least a quarterly basis.

Chain of Custody Forms

A chain of custody (COC) form is used to record sample identification parameters and to document the submission of samples from the field crew to the AgriLife Research Stephenville Laboratory, AgriLife Extension SWFTL, Soil & Aquatic Microbiology laboratory, or EP AREC laboratory staff. Each COC has space to record data for numerous samples. A copy of the COC is found in Appendix C. All entries onto the COC forms will be completed in ink, with any changes made by crossing out the original entry, which should still be legible, and initialing and dating the new entry. After receiving a batch of COCs, the Laboratory Managers open the electronic (Excel) entry table and verifies that all data entered for each sample are correct according to the information on the COC form and data entry sheets. After checking all the data on the forms, the Laboratory Managers initial the forms and sends them to the Project Managers. COCs are kept in three-ring binders in the AgriLife Extension office for at least five years.

AGRILIFE EXTENSION/AGRILIFE RESEARCH Data Errors and Loss

Migration/Transfer/Conversion - File transfer protocols used for ensuring proper exportation of data from the AgriLife Extension database include the data quality assurance procedures integral to the data system.

Backup/Disaster Recovery - The network servers are backed up daily to a tape drive located in a climate controlled, fire-resistant storage area on the AgriLife Research- Stephenville, Texas A&M, and EP AREC campuses. In the event of a catastrophic systems failure, the tapes can be

used to restore the data. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

Archives/Data Retention - Original data recorded on paper files are stored for at least five years. Data in electronic format are stored on tape drives. Complete electronic data sets are archived on tape backup and retained at the Stephenville Research and Extension Center in a fire-resistant storage area.

AgriLife Extension /AgriLife Research Record Keeping and Data Storage

Individual laboratory notebooks, which contain printouts of laboratory data and hand written observations and data, are kept by individual analysts at AgriLife Extension and AgriLife Research. When lab notebooks are filled, they are stored for at least five years by the laboratory manager in hardcopy form. AgriLife Extension / AgriLife Research laboratories keep their electronic data on personal computers for the duration of the project and then in hardcopy files for 5 years after the project. The original field data sheet is filed in a three-ring binder, according to site location and project, and stored for at least five years. COCs and attached documents are stored in numerical order in three-ring binders in the AgriLife Extension Data Manager's office for at least five years. All electronic records are stored for a minimum of five years on personal computers and AgriLife Extension / AgriLife Research fire-resistant cabinets.

Information Resource Management Requirements

Data submitted to TSSWCB will be screened by the AgriLife Extension Quality Assurance Officer prior to submission to ensure that all data records use the proper format and contain all required information. A data review checklist will be utilized (see Appendix D).

Information Dissemination - Submission of the data produced for this project will be transferred from AgriLife Extension / AgriLife Research to TSSWCB as deemed appropriate. Summaries of the data will be presented in the final project report. The TSSWCB may disseminate the validated data and report. The information will also be disseminated through multi-county meetings in the dairy areas of Central Texas, AgriLife Extension publications, and appropriate scientific journals.

C1. ASSESSMENTS AND RESPONSE ACTIONS

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

Table C1-1. Assessments and Response Requirements

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	AgriLife Extension QAO and AgriLife Extension and AgriLife Research project managers	Monitoring of the project status and records to ensure requirements are being fulfilled. Monitoring and review of contract laboratory performance and data quality	AgriLife Extension project managers and EP AREC will report to TSSWCB project manager in quarterly report.
Laboratory Inspections	Annually	TSSWCB QAO	Analytical and QC procedures employed at the laboratory and the contract laboratory	Laboratories have 30 days to respond in writing to the TSSWCB QAO to address corrective actions
Monitoring Systems Audit	Dates to be determined by TSSWCB	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 Extension project managers and EP AREC have 30 days to respond in writing to the TSSWCB QAO to address corrective actions

The commitment to use standard equipment and standard methods for wastewater/manure, water, and soil samples and when producing field or laboratory measurements, requires periodic verification that the equipment and methods are being employed properly. This verification will be provided through an annual field and laboratory performance audit performed by AgriLife Extension QA officer. Individual field personnel will be observed during the actual field investigation to verify that equipment and procedures are properly applied. Any problems that are discovered in the monitoring procedures that would affect the quality of data collected at the demonstration sites will be addressed by the project participants and followed up with a CAR. Follow-up observations will occur within three months when discrepancies are noted. Also, TSSWCB and EPA may conduct a performance audit for this project.

Depending on the analysis, certain methodologies require that standards and reagent blanks be analyzed to verify that no instrument or chemical problem will affect the quality of the data. The specific requirements are presented in Section B5 of the QAPP.

To minimize downtime of all measurement systems, all field equipment and all laboratory equipment must be maintained in a working condition. Also, backup equipment or common spare parts will be available if any piece of equipment fails during use so that repairs or replacement can be made quickly and the sample tasks resumed.

Corrective Action

The AgriLife Extension Project Manager 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 AgriLife Extension project manager. Corrective action documentation will be submitted to the TSSWCB on a quarterly basis with the Progress Report.

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

Corrective actions include identification of root causes and a methodology for correcting the problems. The effect of the problem on the quality of the data is ascertained and documented on the CAR. The programmatic impact (up to and including the removal of data from the database) of the deficiency must be ascertained and documented. The impact of deficiencies must be made on a case-by-case basis in consultation with the AgriLife Extension Project Manager and TSSWCB QAO.

C2. REPORTS TO MANAGEMENT

Laboratory Data Reports

Laboratory data reports contain the results of all specified QC measures listed in Section B5, including but not limited to laboratory duplicates, laboratory control standards, and calibrations. This information is reviewed by the AgriLife Extension QAO and compared to the pre-specified acceptance criteria to determine acceptability of data before submitting it to analyses and storage. This information is also available for inspection by TSSWCB.

Reports to AGRILIFE EXTENSION Project Management

AgriLife Extension / AgriLife Research project participants submit written quarterly progress reports to the AgriLife Extension Project Manager concerning the status of each project task, including data collection activities, for which they are responsible. Any issues or problems associated with the quality of the data are reported to the AgriLife Extension Project Manager through the use of Corrective Action Reports.

Reports to TSSWCB Project Management

Quarterly Progress Report – AgriLife Extension will summarize the AgriLife Extension / AgriLife Research activities for each task; reports problems, delays, and corrective actions; and outlines the status of each task's deliverables.

Monitoring Systems Review Audit Report/Laboratory Audit Report and Response - Following any audit performed by TSSWCB, a report of findings, recommendations and response is sent to the TSSWCB project manager in the quarterly progress report.

Final Project Report - Summarizes AgriLife Extension's and subcontractor activities for the entire project period including a description and documentation of major project activities; evaluation of the project results and environmental benefits; and a conclusion.

D1. DATA REVIEW, VERIFICATION, AND VALIDATION

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

The procedures for verification and validation of data are described in Section D2. The AgriLife Extension Field Supervisors are responsible for ensuring that field data are properly reviewed and verified for integrity. The AgriLife Research / AgriLife Extension Laboratory Managers are responsible for ensuring that analytical laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The AgriLife Extension Project Manager will be responsible for ensuring that all data are properly reviewed and verified, and submitted in the required format to the project database. The AgriLife Extension Project Manger/QAO is responsible for validating the data. Finally, the AgriLife Extension Project Manager/QAO, is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

D2. VERIFICATION AND VALIDATION METHODS

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7 of this document.

Data review, verification, and validation will be performed using self-assessments and peer and management review as appropriate to the project task. The information to be reviewed, verified, and validated (listed by task and responsible party in Table D2-1) is evaluated against technical and project specifications and checked for errors, especially errors in calculations, data reduction, and transcription. Potential errors are identified by examination of documentation and by manual (and computer-assisted) examination of corollary or unreasonable data. If a question arises or an error is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues, which can be corrected are corrected and documented. If an issue cannot be corrected, the AgriLife Extension Project Coordinator consults with the AgriLife Extension / AgriLife Research project participants to establish the appropriate course of action, or the data associated with the issue are rejected. Field and laboratory reviews, verifications, and validations will be documented.

Data validation tasks to be addressed by AgriLife Extension / AgriLife Research include, but are not limited to, the confirmation of lab and field data review, evaluation of field QC results, additional evaluation of anomalies and outliers, analysis of sampling and analytical gaps, and confirmation that all parameters and sampling sites are included in the QAPP. 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. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the AgriLife Extension Project Manager validates that the data meet the data quality objectives of the project and are suitable for reporting to TSSWCB. Pertinent information having to do with inconsistencies with reporting limit specifications; failures in sampling methods and/or laboratory procedures resulting in unavailable data, etc. will be provided on the Data Summary when the data are submitted to TSSWCB.

Table D2-1. Data Review, Verification, and Validation Tasks

Task	Verification	Validation	Responsibility
Field data reviewed for conformance with data collection, sample handling and chain of custody, analytical and QC requirements	Y		AgriLife Extension QAO and Field Operation Supervisor
Post-calibrations checked to ensure compliance with error limits	Y		AgriLife Extension QAO and Field Operation Supervisor
Field data calculated, reduced, and transcribed correctly	Y		AgriLife Extension QAO and Field Operation Supervisor
Laboratory data reviewed for conformance with data collection, sample handling and chain of custody, and analytical and QC requirements to include documentation, holding times, sample receipt, sample preparation, sample analysis, project and program QC results, and reporting	Y		Laboratory Managers
Laboratory data calculated, reduced, and transcribed correctly	Y		Laboratory Managers
Analytical data documentation evaluated for consistency and/or improper practices	Y	Y	Laboratory Managers
Analytical QC information evaluated to determine impact on individual analyses	Y	Y	Laboratory Managers
All laboratory samples analyzed for all parameters	Y	Y	Laboratory Managers
Data set (to include field and laboratory data) evaluated for reasonableness and if corollary data agree	Y	Y	Laboratory Managers, AgriLife Extension QAO
Data review, verification, and validation performed and deviations documented		Y	Laboratory Managers, AgriLife Extension QAO
Outliers confirmed and documented		Y	AgriLife Extension QAO, EP AREC, and AgriLife Extension Project Managers
Field QC acceptable (e.g., field splits)		Y	Laboratory Managers, AgriLife Extension QAO, and AgriLife Extension Project Managers
Sampling and analytical data gaps checked and documented		Y	AgriLife Extension QAO, EP AREC, and AgriLife Extension Project Managers
Verification and validation confirmed. Data meets conditions of end use and are reportable		Y	AgriLife Extension Project Manager

Microsoft Excel will be used for general spreadsheet computation and laboratory control charting of quality control parameters. The AgriLife Extension SWFTL will employ various data handling software on IBM compatible personal computer stations for data on many of the analyzed parameters. Specific software and/or hardware handle data for the different instruments. The AgriLife Extension SWFTL laboratory manager is responsible for review of calculations made by these programs. Soil, water and manure/wastewater quality statistical analyses are performed with SAS programs.

D3. RECONCILIATION WITH USER REQUIREMENTS

The data collected in this project can be used as part of efforts to address nonpoint source pollution issues in impaired watersheds. Samples collected from this project will be analyzed by the AgriLife Extension and AgriLife Research laboratories and reported to TSSWCB for evaluation *E. coli* concentrations in manure/wastewater, water, and in EOF runoff. Data that do not meet requirements will not be submitted to AgriLife Extension or TSSWCB nor will be considered appropriate for any of the uses noted above.

The laboratory manager shall be responsible for reviewing raw data produced by the AgriLife Extension laboratory. The laboratory manager shall check calculations to verify that data are entered into the database correctly and be responsible for internal lab error corrections. CARs will be initiated in cases where invalid or incorrect data have been detected.

Data completeness in this project will be relative to accidents in handling, shipping and laboratory analysis for completeness of the sampling program. It will be the goal of this project to achieve 90 percent completeness; however, statistical analysis will be the final indicator of data validity.

Representativeness and comparability of data, while unique to each individual collection site, is the responsibility of the project manager. By following the guidelines described in this QAPP, and through careful sampling design, the data collected in this project will be representative of the actual field conditions and comparable to similar applications. Representativeness and comparability of laboratory analyses will be the responsibility of the laboratory manager.

Data from the EP AREC laboratories will be reviewed, verified, and validated individually for their ability to meet the data quality objectives of the project. General questions that will be asked, and the metrics on which they will be evaluated, are listed in Table D3-1.

Table D3-1. Methods for Reconciling Results from EP AREC with Data Quality Objectives

Evaluation Issue	Specific Measures
How certain are the source contribution estimates?	laboratory method precision
	laboratory method accuracy
	overall precision of source contribution estimates
	calculated confidence intervals around the average source contribution estimates for each station
What fractions of *E. coli* are from unknown sources?	percentage of unmatched ribotypes and PFGE or ERIC-PCR profiles, and dissimilar ARA profiles
Do the results from ribotyping, PFGE, ERIC-PCR, and ARA agree?	% agreement between ARA, PFGE, ERIC-PCR, and ribotyping result

The project manager will review the final data to ensure that it meets the requirements as described in this QAPP.

APPENDIX A. Work Plan

Monitoring and Educational Programs Focused on *Escherichia coli* Bacteria and Nutrient Runoff on Dairy Operations in the Leon Watershed

Texas State Soil and Water Conservation Board

FY07 CWA Section 319(h)

WORK PLAN

Problem Need/Statement

In 2002, the Leon River below Lake Proctor was listed as being impaired for bacteria according to the Texas Water Quality and 303 (d) lists. Due to the listing for impairment, the Leon River Watershed was selected by the Texas Commission on Environmental Quality (TCEQ) for the development of a TMDL. As of January 2006, the TMDL was in the developmental process. Part of the TMDL development includes modeling the various sources of bacteria in the watershed. However, much of the data used for the model was taken from literature sources due to a lack of actual data from the watershed. The limited data available from the watershed creates challenges in the determination of implementation strategies that will be the most successful in decreasing the amount of bacteria entering surface water in the Leon Watershed.

During the development of the TMDL for the Leon Watershed, livestock and waste application fields were implicated as being significant sources of bacterial loading to the Leon River. Through the finalization of the TMDL and the initiation of the implementation stage, an increased knowledge of the actual levels of bacteria in livestock waste and best management practices that reduce the runoff of bacteria from waste application fields will assist in decreasing movement of bacteria to surface water.

Actual data taken from sources in the Leon Watershed would assist in the development and implementation of the TMDL. Monitoring of bacterial sources listed in the TMDL will be beneficial in determining the sources of greatest bacterial concentrations and will assist in determining the risks associated with a variety of management practices on livestock operations.

Decreasing nutrient and bacteria loads in a watershed is dependent on the education of residents in the watershed. Providing resources to educate residents as to the best management practices that can be used to reduce movement of bacteria to surface waters is essential to the development of a successful TMDL implementation phase. Collection of data in the watershed will provide and increase understanding of bacteria loads in the watershed and will provide knowledge for areas that should be targeted to reduce the risks of bacteria from moving off the land and into surface waters.

General Project Description

The overall objective of the project is to collect watershed specific data in an effort to quantify the major sources of *E. coli* bacteria on dairy operations. Information and data collected during the monitoring phase will be used in the development of an educational program focusing on best management practices (BMPs) to reduce the movement of *E. coli* bacteria and nutrients to surface waters. The

educational program will equip dairy producers with the knowledge and understanding needed to reduce the possibility that their operations will be a source of bacteria and nutrients to the Leon River. The monitoring and the educational programs will be designed to coordinate with the development of a TMDL implementation plan or a watershed plan, and will provide information and assistance for future watershed planning needs.

The objective of the first task is to develop and deliver to TSSWCB the QAPP for EPA approval.

The objective of the second task will be to determine the concentration of *E. coli* bacteria and nutrients present in dairy manure and wastewater throughout first year of the project. Samples of solids and wastewater from dairy lagoons on 4 operations will be collected on a monthly basis. This information will be supplemented with the collection of manure and wastewater samples during field application, up to six times per year, over a 3 year period. The samples will be analyzed to determine the forms and sources of manure containing the greatest bacteria and nutrient concentrations, thus posing the greatest opportunity for improvement in management strategies. (1 month to 36 months)

The goal of the third task will be to assess the concentrations of *E. coli* bacteria and nutrients in surface waters that are located adjacent to waste application fields. Currently, buffer strips are used to reduce the movement of manure and wastewater to surface waters. Monitoring surface waters at upstream and downstream sites will assist in determining if the bacterial and nutrient concentrations are being increased as a result of the waste application field. In particular, application and storm events, up to 10 combined events per year, will be monitored as these events pose the greatest opportunities for movement of bacteria and nutrients to surface water. Grab samples will be collected up (541136°E, 3528813°N and 541664°E, 352947°N) and down stream from the LMU. The LMUs are very close together, therefore there will be only two upstream and downstream sample locations. *E. coli* and nutrients will be determined on upstream and downstream samples collected quarterly from the two selected sites for duration of the project. (6 to 47 months)

The goal of the fourth task is to determine the effectiveness of different BMPs on reducing *E.* coli bacteria and nutrients in runoff from dairy fields.A total of two sites will be selected in Comanche County in the Leon River watershed (Fig. 1) for the evaluation of BMPs. Within each of the two sites, buffer strips, managed and unmanaged, will be established to give a total of 9 plots. The two fields will consist of two manured (wastewater and dry manure) fields (corn, hay, and pasture) and one inorganic fertilized filter strip. The manured fields will have buffer strips, one managed and one unmanaged. Each field will be set up for edge of field monitoring using ISCO samplers. An ISCO will be placed prior to the buffer at the edge of the field and after each buffer, grass buffer strips and riparian buffer strips, at the edge of that land management unit (LMU). Runoff from storm events will be collected by the ISCOs. *Escherichia coli* numbers and nutrients (NO_3-N, P, K, Ca, Mg, Na, and S) will be analyzed from each runoff sample and dissolved oxygen will be analyzed on the grab samples. Bacterial source will be analyzed on selected downstream samples. (12 month to 47 months)

Rainfall simulations will also be conducted on the field and the buffer strips in Task 4. Rainfall simulations will be conducted to measure simulated runoff *E. coli* and nutrient levels from field sites. A Phosphorus Index (PI) will be determined for each of the fields and specific locations within each plot for the simulations will be selected that best represents the PI characteristics and properties upon which the characterization was based. The rainfall simulations will be conducted using a Tlaloc 3000 rainfall

simulator built by Joern's Inc. All rainfall simulation procedures will be conducted in accordance with the Sera-17 National P Project guidelines for rainfall simulations. A total of 4 rainfall simulation replications will be conducted at each of the two fields and filter strips per year. Runoff samples (100 mL) will be collected during each simulation at seven intervals (5, 10, 15, 20, 25, and 30 minutes plus a composite) after runoff is initiated. Each of the timed interval samples will be analyzed for pH and EC in the field, then acidified to pH 2 in the field with HCl. Three composite samples will be collected, one sample for nutrient (P, K, Ca, Mg, Na, and S) analyses except NO_3-N that will be acidified, one for NO_3-N that will not be acidified, and one for *E. coli* that will not be acidified. Cumulative runoff volume will be recorded at one minute increments throughout the 30 minute duration. Water samples will be analyzed for *E. coli* and nutrients as specified above. Soil samples (0-5, 5-15, and 0-15 cm (0-2, 2-6, and 0-6 inches)) will also be collected for each rainfall simulation from each of the four plots. Soil samples will be analyzed for pH; EC; Mehlich-3 P, K, Ca, Mg, Na, and S; and Cd reduction NO_3-N. (20 to 47 months)

The goal of task 5 is the education of dairy producers and the community as to the presence of *E. coli* bacteria and nutrients in manure and wastewater, and BMPs to decrease *E. coli* bacteria and nutrients in runoff from dairy fields. Results of the result demonstrations will be delivered to producers, TCEQ, TSSWCB, and other Texas clientele through Dairy Outreach Program Area meetings and multi-county meetings in the dairy areas of Texas. (12 to 47 months)

Tasks, Objectives, Schedules, and Estimated Costs

Task 1. Development of a QAPP.

Costs: Federal $5,000; Match $3,508 ; Total $8,508

Objectives: To develop a QAPP to outline the protocol and procedures used during the collection and analyses of samples for the entire project. (1 to 6 months)

Subtask 1.1. Develop a QAPP for entire project.

Deliverables:
- QAPP.

Task 2. Evaluation of *E. coli* concentrations in manure and wastewater from dairy operations.

Costs: Federal $121,757; Match $83,348 ; Total $205,105

Objectives: To determine the concentrations of *E. coli* bacteria and nutrients in manure and wastewater on dairy operations throughout a year. (1 to 47 months)

Subtask 2.1. Collection of wastewater samples from dairy lagoons on a monthly basis to determine *E. coli* and nutrient concentrations. Genetic fingerprints of *E. coli* isolates will be compared to a developing statewide source tracking library (AgriLife Research- El Paso AREC). *E. coli* will be isolated from each potential source and fingerprinted using a combination of the enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction technique (ERIC-PCR) and RiboPrinting. Dairy lagoon wastewater samples will also be analyzed for

Bacteroides, the presence of the ruminant genetic marker. Nutrient analyses will include total N using a N analyzer, P, K, Ca, Mg, Na, and S by inductively couple plasma (ICP), pH, and EC. (1 to 36 months)

Subtask 2.2: Collection of dairy wastewater samples at the time of field application to determine *E. coli* and nutrient concentrations. Samples will be collected approximately 6 times per year. (20 to 47 months)

Subtask 2.3: Collection of manure (6 times per year) being applied to the field that has been removed from the facility via a vacuum system and scraped from the lot to determine *E. coli*, and nutrient concentrations. (20 to 47 months)

Deliverables:

•*E. coli* and nutrient concentrations in dairy wastewater over the period of 1 year.
•*E. coli* and nutrient concentrations in fresh dairy manure.
•*E. coli* and nutrient concentrations of dairy manure and wastewater at the time of application.

Task 3. Evaluation of *E. coli* and nutrient concentrations in surface water upstream and downstream of dairy waste application fields.

Costs: Federal $143,8360; Match $97,348 ; Total $241,178

Objectives: To determine the concentration of *E. coli*, bacterial source (selected samples), and nutrients in surface waters at locations upstream and downstream of a waste application field. (20 to 47months)

- **Subtask 3.1**. Collection of surface water samples prior to, during, and after wastewater has been applied to a waste application field bordering a stream segment. Water samples will be grab samples collected from the stream on a monthly basis when water levels allow for sample collection. (20 to 47 months)

- **Subtask 3.2**. Collection of surface water from upstream and downstream sites on a stream segment bordering a waste application field during storm events. Water samples will be grab samples collected from the stream. Samples will be collected for enumeration from 8 to 10 storm events. (20 to 47 months)

- **Subtask 3.3**. *E. coli* will be isolated from edge of field runoff samples, collected after storm events, and upstream and downstream grab samples four times per year. These isolates will be compared to the environmental library from Task 2 to determine the source(s) of the isolates and the relative contribution of each source to the total *E. coli* load. Water samples will also be analyzed for *Bacteroides* human and animal genetic markers. Nutrients, pH, and EC will be analyzed as listed in Subtask 2.1. (20 to 47 months)

Deliverables:

•*E. coli* and nutrient concentrations in surface water from locations upstream and downstream of waste application fields during application events.

•*E. coli* bacterial source (selected samples) and nutrient concentrations in surface water adjacent to waste application fields during storm events.
•Identification of the source(s) contributing *E. coli* to downstream sites.

Task 4. Evaluation of BMPs on loads of *E. coli* and nutrients in runoff from dairy fields.

Costs: Federal $157,770; Match $107,348 ; Total $2653,118

Objectives: To determine the effectiveness of different BMPs on reducing *E. coli* bacteria and nutrients in runoff from dairy fields. (12 to 47 months)

- **Subtask 4.1**. Selection and establishment of 2 field sites with managed and unmanaged buffer strips to give a total of 9 plots, two fields (two manured fields (corn, hay, and pasture) and six buffer strips. (12 to 47 months)

- **Subtask 4.2**. Implement BMPs on the two field sites and collect runoff to estimate the loads of *E. coli* and nutrients in runoff form diary fields. (24 to 47 months)

- **Subtask 4.3**. Conduct yearly rainfall simulations on each of the plots to determine runoff of bacteria and nutrients. Runoff samples will be analyzed according to protocol listed in Subtask 2.1 for nutrients. Soils samples will be collected from each of the rainfall simulations plots (0-2, 2-6, and 0-6) and analyzed for 2:1 water:soil pH and EC; Cd reduction of nitrate-N; and Mehlich-3 by ICP for P, K, Ca, Mg, Na, and S. (20 to 47 months)

Deliverables:

•Evaluation of BMPs on the effectiveness of removing *E. coli* bacteria and nutrients in runoff from dairy fields with different cropping strategies.

Task 5. Education of dairy producers and the community as to the presence of *E. coli* bacteria and nutrients in manure and wastewater, and BMPs to decrease *E. coli* bacteria and nutrients in runoff from dairy fields. (12 to 47 months)

Costs: Federal $10,00; Match $7,019 ; Total $17,019

Objectives: To develop an educational program to relate the information collected during the monitoring and evaluation periods, and to educate producers as to the effectiveness of BMPs on reducing *E. coli* bacteria and nutrients in runoff from dairy fields.

Subtask 5.1. Educational session on *E. coli* bacteria and nutrients present in lagoons and manure and wastewater during application periods. (16 to 47 months)

Subtask 5.2. Field day to show results of BMP implementation on controlling *E. coli* bacteria and nutrients in runoff from dairy fields. (26 to 47 months)

Subtask 5.3. Develop final report and educational materials and publications. (12 to 47 months)

Deliverables:

•Retrospective-post survey to evaluate the effectiveness of the educational program on *E. coli* bacteria and nutrients present in lagoons and manure and wastewater during application periods.
•Survey to evaluate intentions of implementation after the field day to show results of BMP implementation.
•Educational materials and publications.
•Final report.

Coordination, Roles and Responsibilities:

Participating Agencies and Organizations along with their roles in this project include:

- **Texas AgriLife Extension Service. Dr. Sam Feagley**, Extension Soil Environmental Specialist, will coordinate the project as well as assisting in the coordination of the EOF sampling and will coordinate the evaluation of EOF BMPs as well as providing his expertise in soils and manure application systems.
- **Texas AgriLife Extension Service - Dallas. Dr. Ellen Jordan**, Extension Dairy Specialist, will assist in the coordination and evaluation of manure/wastewater sample collection from dairy operations, as well as providing her expertise in dairy management systems.
- **Texas AgriLife Extension Service. Dr. Diane Boellstorff,** Extension Water Quality Specialist, will bring her water quality and watershed expertise to measure the deliverables resulting from management changes.
- **Texas AgriLife Research. Dr. Terry Gentry,** Soil & Aquatic Microbiologist, will bring his knowledge and understanding of microbiology and bacterial source tracking. The laboratory will be responsible for isolation, confirmation, ERIC-PCR fingerprinting, and archival of *E. coli* isolates. Selected isolates will be sent to Dr. Di Giovanni for RiboPrinting.
- **Texas AgriLife Research – El Paso. Dr. George Di Giovanni,** Environmental Microbiologist, will bring is knowledge and understanding of microbiology and bacterial source tracking.
- **Texas AgriLife Research – Stephenville. Dr. Jeff Brady,** Research Scientist, will provide support for sample collection of manure/wastewater from dairy operations, water from EOF, and stream water samples, and will oversee the *E. coli* analysis at the Stephenville laboratory.
- **Texas AgriLife Research – Stephenville.** Nichole Cherry**,** Research Technician, will coordinate the sample collection of manure/wastewater from dairy operations, water from EOF, and stream water samples, and will coordinate the *E. coli* analysis at the Stephenville laboratory.
- **Texas AgriLife Extension Service – Stephenville. Dr. Todd Bilby,** Extension Dairy Specialist, will provide support for sample collection of manure/wastewater from dairy operations, water from EOF, and stream water samples, and will oversee the *E. coli* analysis at the Stephenville laboratory.

Measures of Success:

Determination of the success of the overall project will be through the evaluation of *E. coli* bacteria and nutrient concentrations in water samples entering stream segments in the Leon Watershed that are adjacent to fields receiving manure and wastewater from dairy operations. Although the levels of *E. coli* bacteria and nutrients in the surface water is the ultimate measure of success, this multi-year project will include a combination of monitoring, result demonstrations, BMPs evaluation for effect on *E. coli* and nutrients, and educational activities on which successes will be measured.

The success of the first year of the project will be measured by the development of a database of *E. coli* and nutrient concentrations of manure and wastewater samples. The concentrations of bacteria and nutrients will be related to the source of manure and the weather during the time of collection. In addition, collection of manure during application events will assist in determining the loads of bacteria and nutrients applied to land. The collection of manure and wastewater samples will provide estimates of *E. coli* and nutrient concentrations in different forms of manure to determine if various manure forms pose lesser or greater risks during land application.

The success of the implementation and evaluation of various BMPs effect on *E. coli* and nutrients will help determine the most successful BMPs in mitigating bacteria and nutrient runoff. Thus, recommendations to the producers will be made with percent reductions due to selected BMPs.

Successfulness of educational programs will be determined through the use of a retrospective-post survey tool. The survey tool would allow participants to rate their knowledge of various topics before and after the educational event. The results of the surveys will provide an assessment of increase in knowledge of the program participants.

APPENDIX B. Sampling Process Design and Monitoring Schedule (Plan)

Sample Design Rationale

The sample design is scheduled to provide data to characterize concentrations of *E. coli* in dairy manure/wastewater and in surface water adjacent to fields receiving/having received dairy waste. Changes in *E. coli* and nutrients in the edge-of-field and various matrices associated with dairy waste application will be monitored across various dairy operations and with various BMPs applied to vegetative buffers.

Site Selection Criteria

This project involves the collection of data that will be used to gauge the effectiveness of the vegetative strategies on surface water runoff quality. The data collection effort involves monitoring the *E. coli* and nutrient content of samples associated with various aspects of dairy manure, surface water, and soils rather than sites that are representative of ambient water quality conditions.

Water, Soil, and Manure/Wastewater Samples.

Soil samples in trial and demonstration areas will be collected during the rainfall simulations and will be analyzed using the routine analysis by the AgriLife Extension SWFTL in College Station, TX. Analytes to be analyzed include pH, EC, Ca, Mg, Na, K, P, NO_3-N, and SO_4-S.

Manure/wastewater samples will be collected prior to application to fields if and when this occurs during routine dairy operations. In each sampling event, ten or more sub-samples will be collected and composited into one sample for analysis. The samples will be analyzed by the AgriLife Research Stephenville Laboratory for *E. coli* and by the AgriLife Extension SWFTL in College Station for nutrient analyses. The results of the analysis will aid in making appropriate recommendations regarding dairy manure applications on application fields and in compliance with CNMP provisions.

APPENDIX C. Chain-of-Custody Forms

CHAIN OF CUSTODY RECORD

Project: ***E. coli* Bacteria in the Leon Watershed**							Comments:					
Name and signature of collector:												
Site ID	Sample ID	Sample Type	Container Type	Preserv-ation	Collection Date:	Time:	Num containers	Analyses			Remarks	Tag ID:
								EC	Nutr.	Other		
Relinquished by: (Signature):					Date:	Time:	Received for AgriLife Research Stephenville:				Date:	Time:
Relinquished by: (Signature):					Date:	Time:	Received for AgriLife Extension SWFTL:				Date:	Time:
Relinquished by: (Signature):					Date:	Time:	Received for EP AREC lab:				Date:	Time:
Relinquished by: (Signature):					Date:	Time:	Received for _____ lab:				Date:	Time:
Project Manager (Signature):												

APPENDIX D. Data Review Checklist

Y, N, or N/A

Data Format and Structure

A. Is the file in the correct format? _____
B. Are there any duplicate Tag Id numbers? _____
C. Are the Tag prefixes correct? _____
D. Are all Tag Id numbers 7 characters? _____
E. Are sampling Dates in the correct format, MM/DD/YYYY? _____
F. Is the sampling Time based on the 24 hour clock? (e.g. 13:04) _____
G. Is the Comment field filled in where appropriate? (e.g. unusual occurrence, sampling problems, etc.) _____
H. Are any tag numbers in the Results file that are not in Events file? _____
I. Are confirmed outliers identified with a "1" in the remarks field? _____

Data Quality Review

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

Documentation Review

A. Are blank results acceptable as specified in the QAPP? _____
B. Were control charts used to determine acceptability of field duplicates? _____
D. Were there sampling methods failures and/or deviations from sample design requirements resulting in unreportable data? (explain on next page) _____
E. Were field/laboratory measurement systems failures unresolved, resulting in unreportable data? (explain on next page) _____

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

Date Submitted to TSSWCB:

TAG Series:

Date Range:

Data Source:

Comments:

AgriLife Extension's Data Manager
Signature:_____

Date:

APPENDIX E: Corrective Action Report

Corrective Action Report
CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

Affected sample numbers: _____
Date of sample collection:_____
State the nature of the problem, nonconformance or out-of-control situation:

_____
_____
_____
_____
_____

Possible causes:

_____
_____
_____

Recommended Corrective Actions:

_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:

_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Program Manager:_____

Quality Assurance Officer:_____

APPENDIX F: Field Data Reporting Form

Field Data Reporting Form

		:
Station ID	**Date**	**Time**

Sample ID	**COC Number**

Collector Name(s)

Collecting Agency

Days since last significant rainfall: ☐☐

Weather Observations:

Other Observations:

APPENDIX G: Biosolid Sample Information Form

B 99

BIOSOLID SAMPLE INFORMATION FORM

MANURE, LITTER, AND EFFLUENTS

TEXAS AGRICULTURAL EXTENSION SERVICE
THE TEXAS A&M UNIVERSITY SYSTEM

Soil, Water and Forage Testing Laboratory

Please submit this completed form with samples. Mark each sample bag with your sample identification and ensure that it corresponds with the sample identification written on this form. See sampling procedures and mailing instructions on the back of this form.

SUBMITTED BY:

Name _____ County where sampled _____

Address _____ Phone _____

City _____ State _____ Zip _____

FOR: (Will not receive copy)

Name _____

Address _____

City _____ State TX Zip _____

Payment *(DO NOT SEND CASH).*

- ☐ Check
- ☐ Money Order
- ☐ Government Account

Amount Paid $ _____

SAMPLE I.D.			SAMPLE INFORMATION (Required)		(See options listed below)
Laboratory # (For Lab Use)	Your Sample I.D.	Quantity Represented	Biosolid to be applied to: (cultivated crop land, non cultivated crop land, or other)	Sample Type: Please check box	Requested Analyses
				☐ solid manure ☐ litter ☐ liquid-effluent ☐ other_____	☐ 1 manure/litter ☐ 2 effluent and other
				☐ solid manure ☐ litter ☐ liquid-effluent ☐ other_____	☐ 1 manure/litter ☐ 2 effluent and other
				☐ solid manure ☐ litter ☐ liquid-effluent ☐ other_____	☐ 1 manure/litter ☐ 2 effluent and other
				☐ solid manure ☐ litter ☐ liquid-effluent ☐ other_____	☐ 1 manure/litter ☐ 2 effluent and other

Please select one analysis group per sample:

Analysis for Biosolids

1. **Nitrogen + Minerals + % Moisture** — **$15 per sample**
   (N, P, K, Ca, Mg, Na, Zn, Fe, Cu, Mn and % moisture)
   (for solid manures only)

2. **Nitrogen + Minerals** — **$15 per sample**
   (N, P, K, Ca, Mg, Na, Zn, Fe, Cu, and Mn.
   (for effluents and other misc. samples)

Please follow all sampling and shipping instructions on back of of this form.

Form B1, 11-99

APPENDIX H: Water Sample Information

AgriLIFE EXTENSION
Texas A&M System

Soil, Water and Forage Testing Laboratory
Department of Soil and Crop Sciences
Texas AgriLife Extension Service

D-617a
W 08

WATER SAMPLE INFORMATION FORM

Please submit this completed form and payment with samples. Mark each sample bottle with your sample identification and ensure that it corresponds with the sample identification written on this form. *See sampling and mailing instructions on the back of this form.
(PLEASE DO NOT SEND CASH)

SUBMITTAL AND INVOICE INFORMATION: This information will be used for all official invoicing and communication.

Name ______________________________ County where sampled ____________________

Address ______________________________ Phone ____________________

City ____________________ State______ Zip ______ ____________________

CLIENT NAME: Client name will only be included with information above on result reports.

Name ______________________________

Lab Use only

Payment (DO NOT SEND CASH)
☐ Check
☐ Money Order
☐ Credit Card – requires additional form*

Amount Paid $____________________
Make Checks Payable to: **Soil Testing Laboratory**
*Credit card payment forms can be downloaded at http://soiltesting.tamu.edu

Sample ID **SAMPLE INFORMATION (Required)** **(see options listed below)**

Laboratory # (For Lab Use)	Your Sample ID		Water Source	Water Use:		Requested Analyses
		☐ Public ☐ Private	☐ Well ☐ Other ☐ Pond ☐ Lake ☐ Steam ☐ Processing Plant ☐ Animal Feedlot ☐ Wastewater treatment	☐ Aquaculture ☐ Commercial ☐ Domestic ☐ Greenhouse ☐ Hydroponics ☐ Irrigation-forage ☐ Irrigation-ornamentals	☐ Irrigation-turf ☐ Irrigation-vegetables ☐ Livestock ☐ Recreation ☐ Wasterwater ☐ Other	☐1 ☐2 ☐3 ☐4 ☐ ☐ ☐
		☐ Public ☐ Private	☐ Well ☐ Other ☐ Pond ☐ Lake ☐ Steam ☐ Processing Plant ☐ Animal Feedlot ☐ Wastewater treatment	☐ Aquaculture ☐ Commercial ☐ Domestic ☐ Greenhouse ☐ Hydroponics ☐ Irrigation-forage ☐ Irrigation-ornamentals	☐ Irrigation-turf ☐ Irrigation-vegetables ☐ Livestock ☐ Recreation ☐ Wasterwater ☐ Other	☐1 ☐2 ☐3 ☐4 ☐ ☐ ☐
		☐ Public ☐ Private	☐ Well ☐ Other ☐ Pond ☐ Lake ☐ Steam ☐ Processing Plant ☐ Animal Feedlot ☐ Wastewater treatment	☐ Aquaculture ☐ Commercial ☐ Domestic ☐ Greenhouse ☐ Hydroponics ☐ Irrigation-forage ☐ Irrigation-ornamentals	☐ Irrigation-turf ☐ Irrigation-vegetables ☐ Livestock ☐ Recreation ☐ Wasterwater ☐ Other	☐1 ☐2 ☐3 ☐4 ☐ ☐ ☐

Describe any problems you have observed to want to correct:

1. Routine Analysis (R) (Conductivity, pH, Na, Ca, Mg, K, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-, B, Nitrate-N. Hardness, and SAR)	**$20 per sample**
2. R + Metals In addition to Routine Analysis includes: (Zn, Fe, Cu, Mn and total P)	**$30 per sample**
3. R + Titrate of Drip Irrigation	**$25 per sample**
4. R + Metals + Titrate for Drip Irrigation	**$35 per sample**

Form W8.08

F

orm

APPENDIX I: Soil Sample Information Form

AgriLIFE EXTENSION
Texas A&M System

Soil, Water and Forage Testing Laboratory
Department of Soil and Crop Sciences
Texas AgriLife Extension Service

D-494
S 08

SOIL SAMPLE INFORMATION FORM

Please submit this completed form and payment with samples. Mark each sample bag with your sample identification and ensure that it corresponds with the sample identification written on this form. *See sampling and mailing instructions on the back of this form.
(PLEASE DO NOT SEND CASH)

SUBMITTAL AND INVOICE INFORMATION: This information will be used for all official invoicing and communication.

Name ______________________ County where sampled ______________

Address ______________________ Phone ______________

City ______________ State______ Zip ______

CLIENT NAME: Client name will only be included with information above on result reports.

Name ______________________

Lab Use only

Payment (DO NOT SEND CASH)
☐ Check
☐ Money Order
☐ Credit Card – requires additional form*

Amount Paid $______________
Make Checks Payable to: **Soil Testing Laboratory**
*Credit card payment forms can be downloaded at http://soiltesting.tamu.edu

Sample ID — SAMPLE INFORMATION (Required) (see options listed below)

Laboratory # (For Lab Use)	Your Sample ID	Acreage Represented	Previous lime/ Fertilizer	What are you growing?	Requested Analyses	How is Forage Used?
					☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9	☐Grazing (G) ☐G&H ☐ hay (H) ☐ *Min. requirement
					☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9	☐Grazing (G) ☐G&H ☐ hay (H) ☐ *Min. requirement
					☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9	☐Grazing (G) ☐G&H ☐ hay (H) ☐ *Min. requirement
					☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9	☐Grazing (G) ☐G&H ☐ hay (H) ☐ *Min. requirement
					☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9	☐Grazing (G) ☐G&H ☐ hay (H) ☐ *Min. requirement
					☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9	☐Grazing (G) ☐G&H ☐ hay (H) ☐ *Min. requirement

Describe any problems you have observed to want to correct:

1. Routine Analysis (R) (pH, NO3-, P, K, Ca, Mg, Na, S and Conductivity)	$10 per sample
2. R + Micronutrients (Micro) (Zn, Fe, Cu, and Mn)	$15 per sample
3. R + Micro + Boron (B	$20 per sample
4. R + Detailed Salinity (Sal) (TDN and energy calculated)	$25 per sample
5. R + Micro + Sal	$30 per sample
6. R + Micro + Detailed Lime Requirement (Lime)	$20 per sample
7. R + Micro + B + Lime + Organic Matter + Sal	$50 per sample
8. R + Textural Analysis	$20 per sample
9. R + Organic Matter	$20 per sample

Note: Organic Matter, Detailed Salinity and Texture may require longer processing time.

Form 82-08

APPENDIX J: Example Letter to Document Adherence to the QAPP

TO: (name)
(organization)

FROM: (name)
(organization)

Please sign and return this form by (date) to:

(address)

I acknowledge receipt of the referenced document(s). I understand the document(s) describe quality assurance, quality control, data management and reporting, and other technical activities that must be implemented to ensure the results of work performed will satisfy stated performance criteria.

_____

Signature Date

APPENDIX K: Soil, Effluent, and Stream Water Sampling and Analysis Procedures

Soil Sampling and Analysis Procedures

Texas AgriLife Extension Service

Dr. Sam Feagley

Sampling Procedures:

Approximately 10 to 15 random soil subsamples will be collected on each of the small rainfall simulation plots at depths of 0-2, 2-6, and 0-6 inches and placed into a soil sample bag per depth and per plot, giving four replications for each rainfall simulation site. Composite soil samples will be collected from each of the fields used in the study as part of the Phosphorus Index requirements. There will be 15 subsamples composited for every 10 to 40 acres according to AgriLife Extension SWFTL recommended soil sampling techniques collected at 0-2, 2-6, and 0-6 inches. These sampling depths meet the Texas CAFO rule depth requirements for Zone 1, regardless of the land use. The best professional judgment technique will be used to collect the 15 subsamples per area (TCEQ, 2003).

Effluent, upstream and downstream water samples will be collected using a 6 to 10 foot aluminum pole with a clean plastic bottle taped to the end. The container will be held under the water at least 6 inches to avoid the surface. Approximately 10 to 15 subsamples will be collected in a clean plastic bucket, mixed thoroughly and subsamples collected for nutrient and bacteria analyses (Provin, 2003).

Solid manure samples will be collected with clean sampling tools such as a shovel or soil sampling tube. Approximately 10 to 15 subsamples will be collected from each sampling location. The subsamples will be mixed thoroughly in the field and subsamples will be collected for nutrient and bacteria analyses (Provin, 2003).

Sample Handling, and Preparation and Storage:
Soil samples will be stored in the truck until returned to the College Station for a minimum of one day and a maximum of four days. Once the samples are returned to College Station, the samples will be dried in a forced air oven at 90ºC until dry, pulverized to pass through a 60 mess sieve, and stored at room temperature until various extractions are made (Provin, 2003). Soils will be weighed for each extraction in the lab used by Dr. Sam Feagley, then transported to the AgriLife Extension SWFTL after the extractions have been made for final analyses.

Effluent, upstream and downstream water samples will be collected in 500 mL wide mouth plastic containers and placed into an ice chest immediately after mixing in the field. The samples will be kept in the ice chest at 4^{o}C for a minimum of one day and a maximum of four days before being transferred to a refrigerator until analyses are completed.

Solid manure samples will be collected in 500 mL wide mouth plastic containers and placed into an ice chest immediately after mixing in the field. The samples will be kept in the ice chest at $4^{o}C$ for a minimum of one day and a maximum of four days before being transferred to a refrigerator until analyses are completed.

References:

Provin, Tony. 2003. Texas Cooperative Extension Soil, Water and Forage Laboratory. Standard operating procedures.

TCEQ Regulatory Guidance, Water Quality Division. 2003. Soil sampling for nutrient management plans. RG-408.

APPENDIX L: ERIC-PCR Laboratory Protocol

Laboratory Protocol for Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) Fingerprinting of *Escherichia coli*

1. Select isolated colonies from overnight cultures of *E. coli* isolates on TSA plates.

2. Transfer colonies to sterile microfuge tubes 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 follows:
   ERIC-PCR Master Mix

MASTER MIX	Amt (uL)	Final Calc	Final Units
dH2O	**31.5**		
10X PCR buffer I w Mg	**5**	1	X
20 mM dNTP	**0.5**	200	uM each
ERIC Primer Mix	**5**	600	nM each
BSA (30 mg/ml)	**2.5**	1.5	ug/uL
AmpliTaqGold (Units)	**0.5**	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 TBE buffer and 5.0 g agarose. Microwave until agarose is fully dissolved, tighten cap, then place in agarose in a 50°C water bath.

10. Assemble gel casting tray with 30-tooth, 1 mm thick comb.

11. Remove the agarose from the water bath, gently mix by swirling (avoiding bubbles) and pour into the gel casting tray.

12. Allow gel to solidify for approximately 30 minutes, carefully remove comb, then 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.

13. The following items will be needed for electrophoresis:

    100 bp ladder (0.5 µg/10 µl) (1000 µl final, enough for 100 lanes)

    200 µl Roche (Cat. #1721933) 0.25 µg/µl 100 bp ladder stock

    166 µl 6X ERIC-PCR loading buffer (see below)

    100 µl 10X PCR buffer

    534 µl molecular grade water

    Store in cold room

    6X ERIC-PCR Loading Buffer

    25 mg bromphenol blue (0.25%)

    25 mg xylene cyanol (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

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

15. Load the gel in the cold room as follows (max. of 22 samples per gel):

    a. Load 10 µl of ERIC-PCR Blank into the first two lanes

    b. Load 10 µl of 100 bp ladder (0.5 µg) into third lane on gel

c. Load 10 µl of PCR reactions into next 8 lanes
d. Load 10 µl of 100 bp ladder (0.5 µg) into lane 12
e. Load 10 µl of PCR reactions into next 8 lanes
f. Load 10 µl of 100 bp ladder (0.5 µg) into lane 21
g. Load 10 µl of PCR reactions into next 6 lanes
h. Load 10 µl of 100 bp ladder (0.5 µg) into lane 28
i. Load 10 µl of ERIC-PCR Blank into the last two lanes

If running a gel with fewer samples, follow steps above until last sample, followed by one lane with ladder and load ERIC-PCR Blank into remaining lanes on gel.

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

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

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

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

20. Place gel on UV transilluminator and photograph using AlphaImager software. Save digital photograph as a TIFF file (default) and print a hardcopy for notebook.

APPENDIX M: Automated Ribotyping Laboratory Protocol

Laboratory Protocol for Automated Ribotyping of *Escherichia coli* Using the DuPont Qualicon RiboPrinter

Storing and Handling Disposables

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

Heating 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 16-24 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 (Brain-Heart Infusion) 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.

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.

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

2. Transfer Sample Buffer to Intermediate Tubes

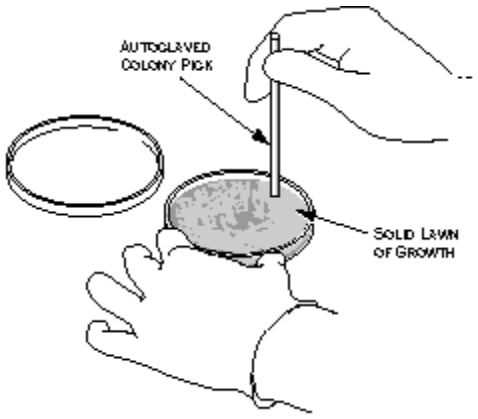
a) Locate the 250 mL twist-top bottle of sample buffer supplied in Pack # 1 Install the twist cap.
b) Transfer about 5 mL of buffer to a sterilized disposable 15 mL intermediate working tube.

3. Add sample buffer to microcentrifuge tubes

1. Place a sterile 0.65 mL microfuge tube in each of the eight holes in the lower row of the sample preparation rack.
2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.
   For Gram positive samples (e.g. *S. aureus* and *L. innocua* QC strains), add 40 µL of sample buffer.
3. Close the lids on the tubes.

4. Harvest the Samples

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



2. For Gram negative samples (e.g. *E. coli*), perform 1 pick placed into 200 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*.

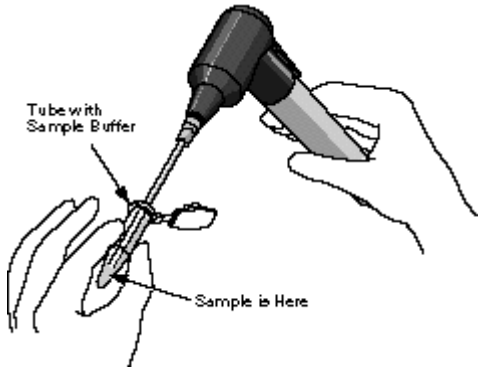
5. Mix the Samples

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

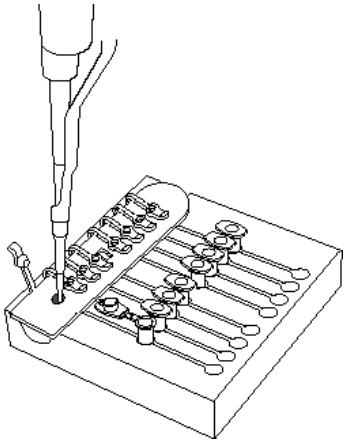
1. Making certain not to touch the sample end of the pick, place the pick into one of the filled sample tubes.
2. While holding the tube with the open end facing away from you, carefully attach the pick to the hand-held mixer. The fit of the pick in the coupling will be loose.

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.

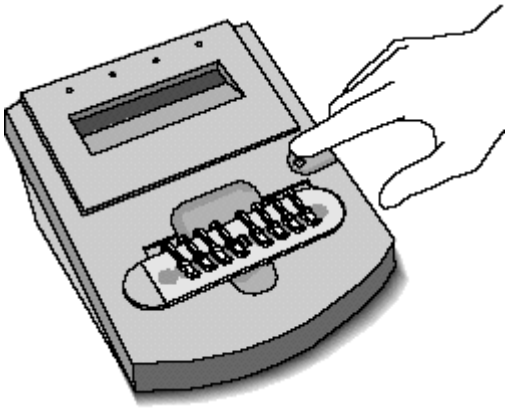
2. Using a 100 µL pipetter, pipette 30 µL of sample from the microcentrifuge tube into the well.
3. Close the lid cover for the well.
4. Repeat for remaining samples using a new pipette tip for each sample.

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.
2. 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) for 1 week at 4 °C, or for several months at -70 °C.

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

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

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

Creating and Loading a Batch

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

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

You can also create special batches:

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

From the Instrument Control Base Window:

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

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

4. To enter information about the sample, click on the **View** button with the mouse button, then click on **Sample Items**. Click on the options you want to display.
5. Enter your initials and any comment you want to record about the batch.
6. Select the lot number fields and record for all reagents.

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

7. For each well in the sample carrier, choose the type (Sample or Control [QC Number]) from the Sample Type field. The system defaults to Sample.
8. Once you define the Sample Type as Sample, type in the name you actually want to use. This information will appear as Sample Label in the Data Analysis software screens.
9. You can change the RiboGroup library name if needed. Do this by clicking on the button next to the field with the mouse button. A pop up menu appears listing your choices. If you want to add a new library name, move the pointer to the line and click with the

mouse button to get a cursor, then type in the new library name. Once you have saved this file, the new name will be added to the pop up list for future use. Do **NOT** change the DuPont ID field. If you select one of the QC strains, the system automatically enters QC in the DuPont ID and RiboGroup Library fields. Do not change these names. If you wish, you may enter a name for the Custom ID library.

10. Repeat for the other seven samples.
11. Click on Save and Submit Batch to Instrument.

Loading Disposables

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

CAUTION! *Do not try to move the pipette manually. You will cause the system to lose the step count. This can result in the loss of batch data. If the pipette is blocking the S/T chamber that you are instructed to use, STOP. Call Customer Support.*
CAUTION! *Do not load disposables until you are prompted by the system. If you try to load them earlier, the alarm will sound as long as the doors are open. If you do load disposables ahead of time, the MP Base will be moved to the wrong position and you will not be able to begin processing the batch. You will not be able to move the MP base manually.*

1. Check the DNA Preparation Waste Container

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

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

2. Load the Sample Carrier

1. Place the sealed carrier into the labeled slot on the far right of the characterization unit.
2. Push the sample carrier down firmly until it snaps into place.

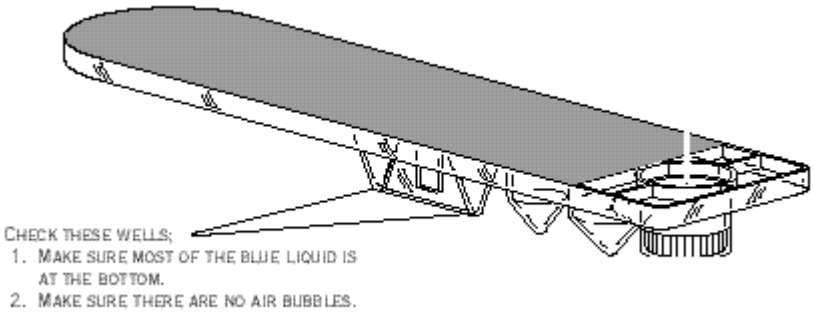
CAUTION! Place the rounded edge of the sample carrier on your right as you view the characterization unit. Position the carrier this way to insure correct identification of the sample wells.

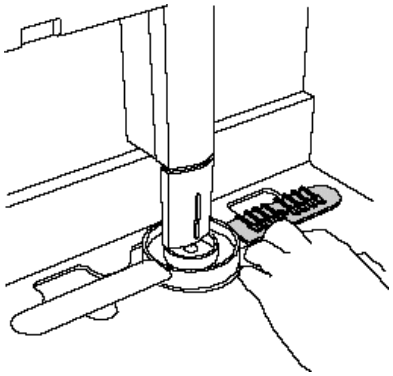
3. Load the DNA Prep Carrier

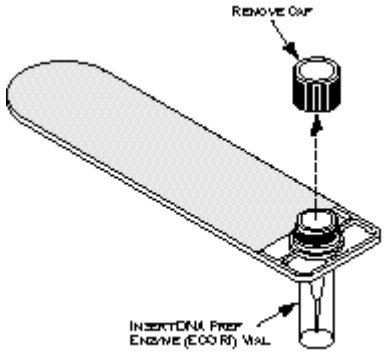
1. Remove the DNA Prep carrier from the refrigerator.
2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**
3. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.
4. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as a 50 U/µL working stock as follows.**

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

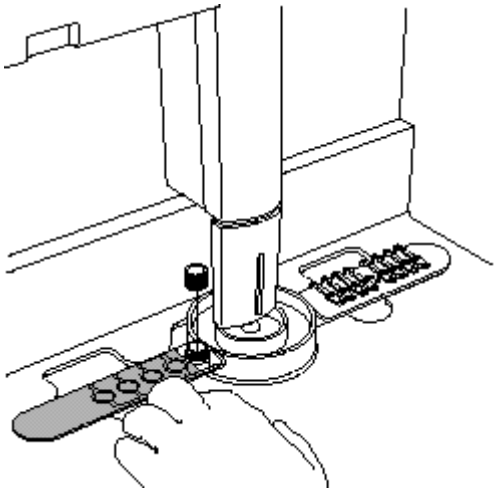
   During addition of the Buffer, mix enzyme and buffer to homogeneity by stirring with the micropipette tip.





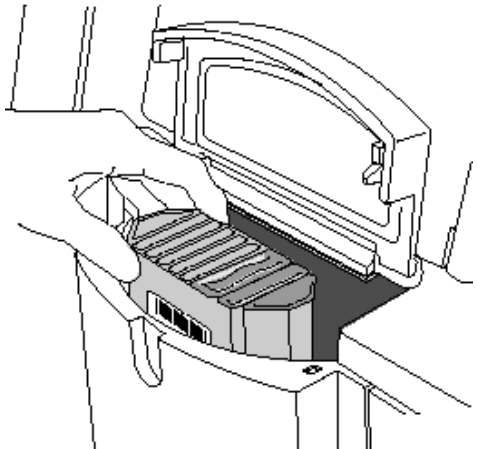


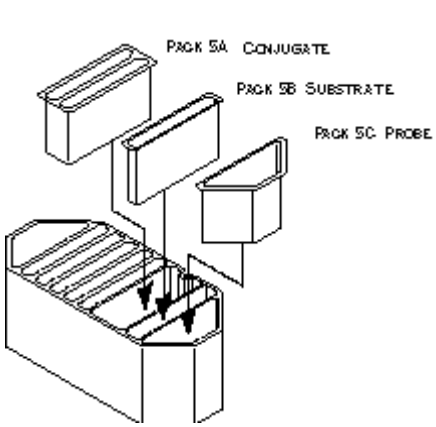
5. Remove the cap from the Enzyme vial.
6. Insert the vial into the carrier.
7. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.
8. Push the DNA Prep carrier down firmly until it snaps into place.

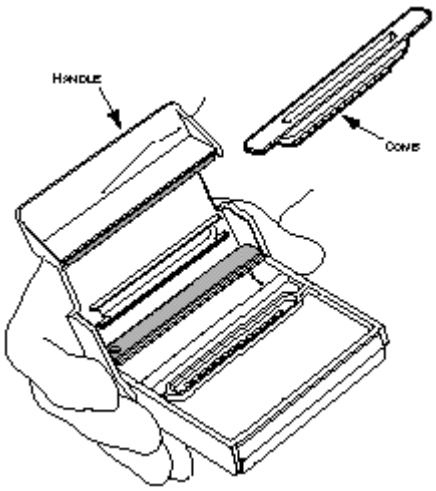


4. Load the MP Base and Carousel

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.

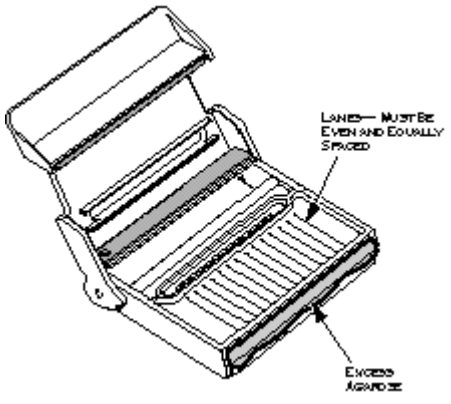
CAUTION! Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.

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.

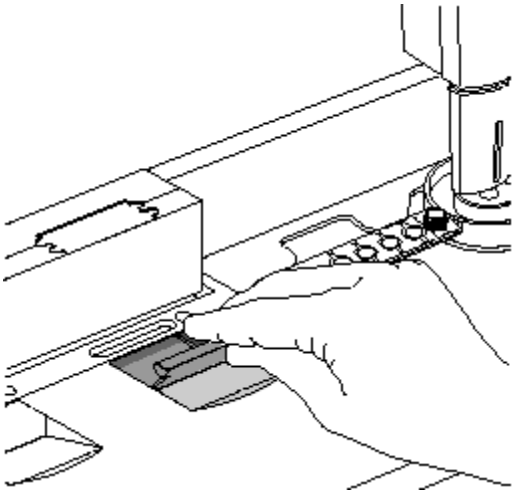
3. Unfold the handle of the cassette towards you until the handle snaps into place.
4. Check the front edge of the gel cassette and the lanes of the gel.

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.

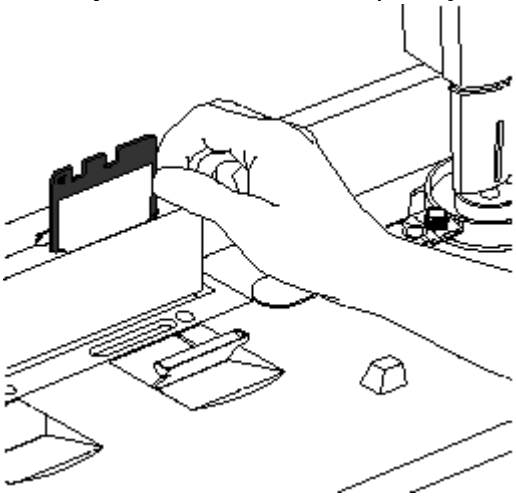


6. Press the cassette forward firmly until it snaps into place.

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.

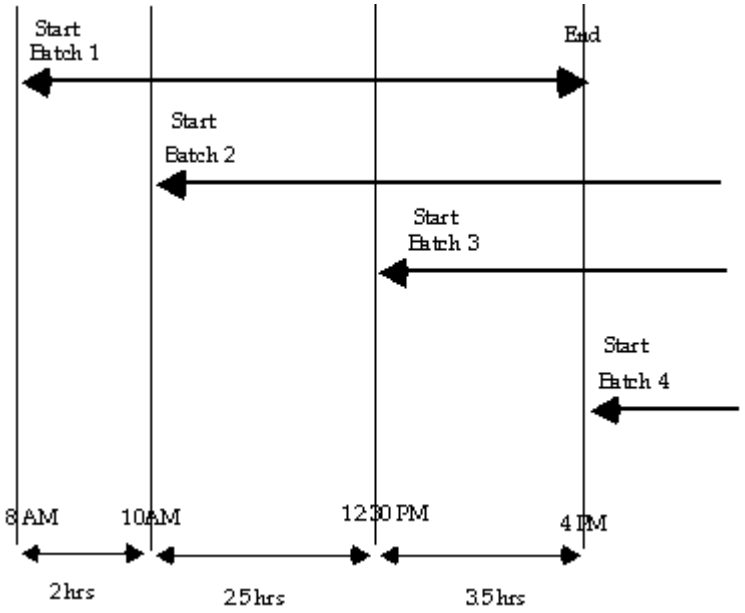
8. Load the Next Batch

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.

APPENDIX N: *E. coli* Archival Protocol

Laboratory Protocol for 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.

1. Select a well isolated colony of purified *E. coli*.

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. Verify that the cells have been resuspended.

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

APPENDIX O: *E. coli* Isolation Laboratory Protocol

Laboratory Protocol for Isolation of *Escherichia coli* from Water Samples

1. Follow the EPA Modified mTEC procedure described in "Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*" (EPA/821/R-97/004, Modified EPA Method 1103.1; http://www.epa.gov/nerlcwww/RecManv.pdf).

2. After the Modified mTEC 44.5±0.2°C incubation, the plates should be immediately stored at 4°C until shipment to prevent growth of non-*E. coli* coliforms on the plates.

3. Plates with red or magenta colored colonies should be parafilmed or taped closed, placed in plastic bags and then secured with tape to prevent the plates from being disturbed during shipment.

4. Ship plates in insulated coolers with ice packs sufficient to keep the plates between 1–4°C and ship by next day courier to:

   Dr. Terry Gentry
   Texas A&M University
   Soil & Crop Sciences
   370 Olsen Blvd, Heep Center Rm 539
   College Station, TX 77843 979-845-5323

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

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

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

7. Invert and incubate plates at 35–37°C for 20–24 h.

8. 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.

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