

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

**Development of an *E. coli* Bacterial Source Tracking Library and  
Assessment of Bacterial Sources Impacting Lake Waco and Lake  
Belton**

**Quality Assurance Project Plan**

**Texas State Soil and Water Conservation Board**

prepared by

Parsons

Texas A&M El Paso Agricultural Research and Extension Center  
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Effective Period: September 1, 2004 to August 31, 2005

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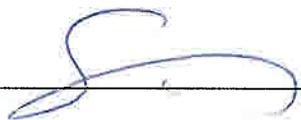
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**Section A1: Approval Sheet**

Quality Assurance Project Plan for Development of an *E. coli* Bacterial Source Tracking Library and Assessment of Bacterial Sources Impacting Lake Waco and Lake Belton

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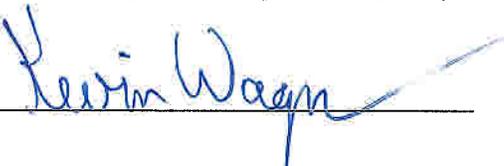
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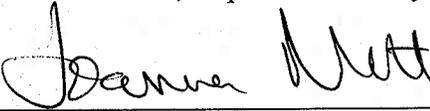
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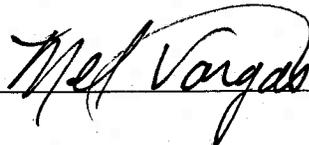
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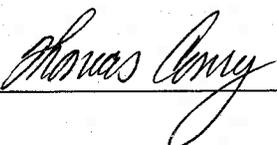
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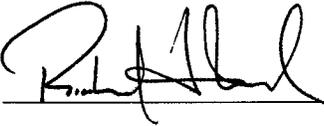
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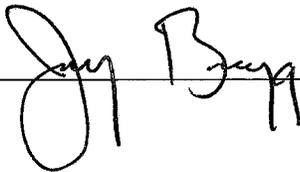
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Signature:  Date: 7 June 2005

**Brazos River Authority (BRA)**

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## List of Acronyms and Abbreviations

ACS	American Chemical Society
ARA	antibiotic resistance analysis
BMP	best management practices
BRA	Brazos River Authority
BST	bacterial source tracking
CAR	corrective action report
CDC	Center for Disease Control
CFU	colony forming units of bacteria
COC	chain of custody
CR	county road
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DQO	data quality objectives
EP AREC	Texas A&M El Paso Agricultural Research and Extension Center
EPA	United States Environmental Protection Agency
ERIC-PCR	enterobacterial repetitive intergenic consensus sequence polymerase chain reaction
FM	Farm to Market Road
GPS	global positioning system
ID	identification
LIMS	laboratory information management system
MAL	minimum analytical level
MPN	most probable number
NCCLS	National Committee for Clinical Laboratory Standards
NIST	National Institute of Standards and Technology
NPS	nonpoint source
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PM	project manager
QA	quality assurance
QAO	quality assurance officer
QAPP	quality assurance project plan
QC	quality control
RPD	relative percent deviation
SOP	standard operating procedure
SH	state highway
SM	Standard Methods for the Examination of Water and Wastewater
TAMU	Texas A&M University (College Station campus implied)
TAMU-CC	Texas A&M University – Corpus Christi
TBD	to be determined
TCEQ	Texas Commission on Environmental Quality
TFB	Texas Farm Bureau

TMDL  
TSSWCB  
USEPA

total maximum daily load  
Texas State Soil and Water Conservation Board  
United States Environmental Protection Agency

### **Section A3: Distribution List**

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

#### **United States Environmental Protection Agency, Region VI**

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Title: Regional Environmental Planner

## **Section A4: Project/Task Organization**

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

**USEPA** – Provides project overview and funding at the Federal level.

Randall Rush, USEPA Texas Nonpoint Source Project Manager

Responsible for overall performance and direction of the project at the Federal level. Ensures that the project assists in achieving the goals of the federal Clean Water Act (CWA). Reviews and approves the quality assurance project plan (QAPP), project progress, and deliverables.

**TSSWCB** - Provides project overview and funding at the state level.

Kevin Wagner, TSSWCB Project Manager and Quality Assurance Officer

Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Responsible for determining that the project meets the requirements for planning, quality control (QC), quality assessment, and reporting under the CWA Section 319 program. Provides the primary point of contact between the Texas State Soil and Water Conservation Board (TSSWCB) and the Texas A&M El Paso Agricultural Research and Extension Center (EP AREC) and Texas Farm Bureau (TFB) contractors. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified. Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB and USEPA participants. Responsible for verifying that the QAPP is followed by TAMU and TFB participants. Conveys QA problems to appropriate TSSWCB management. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures.

**EP AREC** – Responsible for isolation and purification of *E. coli* from fecal specimens and water samples, archival of *E. coli* cultures, ERIC-PCR screening and ribotyping of *E. coli* isolates, data analysis, and reporting tasks for the project including (together with Parsons) development of data quality objectives (DQOs) and a quality assurance project plan (QAPP). EP AREC will be responsible (with the TFB and Parsons) for coordination, development, and delivery of quarterly reports and the final project report.

Dr. George D. Di Giovanni, EP AREC Project Manager and Laboratory Supervisor

Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract work plan and in the QAPP; assessing the quality of sub-participant work; submitting accurate and timely deliverables to the TSSWCB Project Manager; and coordinating attendance at conference calls, training, meetings, and related project activities with the TSSWCB. 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. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions.

**TAMU** – Responsible for pulsed-field gel electrophoresis (PFGE) analysis of *E. coli* isolates provided by EP AREC. Responsible for PFGE data analysis and QA procedures for that task. TAMU will contribute to the development of quarterly reports and the final project report.

Dr. Suresh D. Pillai, TAMU Laboratory Supervisor

Responsible for laboratory supervision and technical direction for pulsed-field gel electrophoresis (PFGE) analysis of *E. coli* isolates provided by EP AREC. Responsible for verifying that the QAPP is followed by the TAMU 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 TAMU laboratory. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions. Responsible for submitting accurate and timely data analyses and contributions for progress and final reports to the EP AREC Project Manager.

**TAMU-CC** – Responsible for ARA of *E. coli* isolates provided by EP AREC. Responsible for ARA data analysis and QA procedures for that task. TAMU-CC will contribute to the development of quarterly reports and the final project report.

Dr. Joanna B. Mott, TAMU-CC Laboratory Supervisor

Responsible for laboratory supervision and technical direction for ARA of *E. coli* isolates provided by EP AREC. Responsible for verifying that the QAPP is followed by the TAMU-CC 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 TAMU-CC laboratory. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions. Responsible for submitting accurate and timely data analyses and contributions for progress and final reports to the EP AREC Project Manager.

**Texas Farm Bureau** – Responsible (with EP AREC) for coordination, review, and delivery of quarterly reports and the final project report.

Ned Meister, Texas Farm Bureau Project Manager

Responsible for ensuring that tasks and other requirements in the contract are executed on time and as defined by the grant work plan; assessing the quality of work by sub-participants; submitting accurate and timely deliverables and costs to the TSSWCB PM; and coordinating attendance at conference calls, training, meetings, and related project activities with the TSSWCB.

**City of Waco** – Responsible for the culturing and enumeration of *E. coli* in ambient water samples, and providing those cultures to the EP AREC laboratory. Assists in the collection of water samples from Lake Waco and its tributaries.

Tom Conry, City of Waco Project Manager

Responsible for supervision and technical direction of culturing and enumeration of *E. coli* in ambient water samples. Responsible for verifying that the QAPP is followed by the City of Waco 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 City of Waco laboratory. Responsible for coordination with the Parsons Project Manager on ambient water sample collection. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions. Responsible for submitting accurate and timely contributions for progress and final reports to the TFB Project Manager.

**Brazos River Authority** – Assists in the collection of water samples.

Kyle Headley, BRA Project Manager

Responsible for ensuring the collection of ambient water samples in the Lake Belton watershed, delivery of those samples to the City of Waco laboratory, and coordination with the City of Waco and Parsons project managers on sample collection. Responsible for verifying that the QAPP is followed by BRA staff, 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 BRA. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions. Responsible for submitting accurate and timely contributions for progress and final reports to the TFB PM.

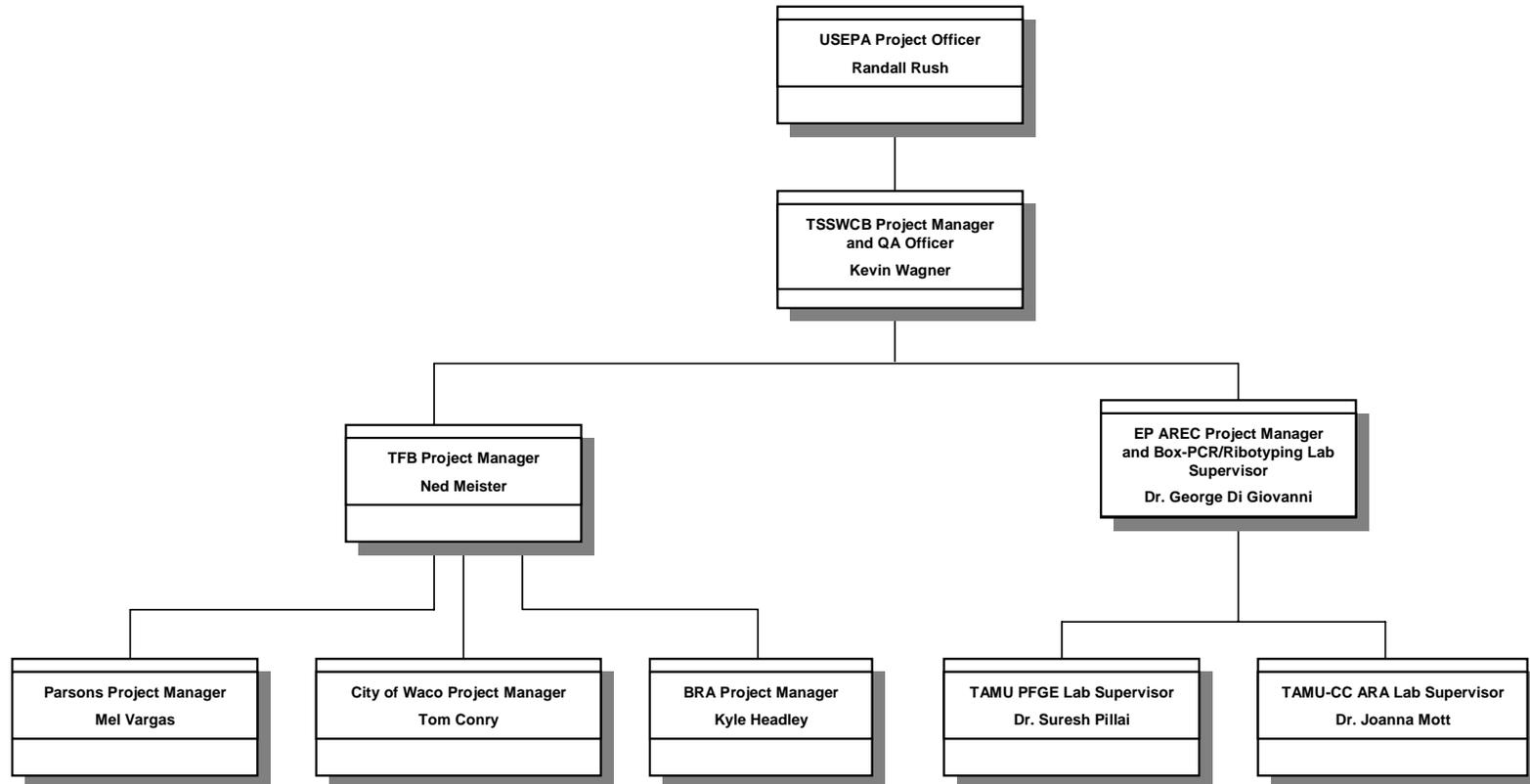
**Parsons** – Responsible for performing a sanitary survey of the watershed to identify potential fecal sources, collection of fecal samples from known source samples, collection of ambient

water samples (with the BRA and City of Waco), data analysis and interpretation (with EP AREC), and developing a final report to the TFB and TSSWCB (with EP AREC).

Mel Vargas, Parsons Project Manager

Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract work plan and in the QAPP; submitting accurate and timely deliverables and costs to the TFB PM; and coordinating attendance at conference calls, training, meetings, and related project activities with the TFB and TSSWCB. Responsible for supervising the watershed sanitary survey, collection of known source library samples, collection of ambient water samples, data interpretation, and preparation of reports. Responsible for verifying that the QAPP is distributed and followed by Parsons personnel, 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 Parsons. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions.

**Figure A4-1. Project Organization Chart**



## Section A5: Problem Definition/Background

Protection of our water resources is one of the most significant environmental challenges of the new millennium. Nonpoint sources (NPS) of pollution, especially from agricultural activities, can greatly impact water quality. One key component in effectively implementing a NPS pollution management program is the identification and assessment of sources of bacterial contamination, especially for impaired waterbodies on the State of Texas §303(d) list such as those in the Lake Waco and Lake Belton watersheds. Proper evaluation of these sources is needed to develop microbial total maximum daily loads (TMDLs) and best management practices (BMPs). This information may also be useful to properly assess risk in contact recreation, as many waterborne pathogens causing human illness do not colonize nonhuman hosts.

Fecal coliform bacteria have extensively been used as an indicator of fecal pollution and the potential presence of other pathogenic microorganisms in water. It has been established that the fecal coliform bacterium *Escherichia coli* (*E. coli*) is more closely associated with fecal pollution than other fecal coliform bacteria, which may normally reside and multiply in the environment. *E. coli* is a common inhabitant of animal and human intestines and recent studies have shown that isolates from humans and various host animals (e.g. cattle, chickens, and pigs) may differ genetically and phenotypically. Use of genetic and biochemical tests may allow the original host animal to be identified, referred to as bacterial source tracking (BST). Molecular tools appear to hold the greatest promise for BST, providing the most conclusive characterization and level of discrimination for isolates. Of the molecular tools available, ribosomal ribonucleic acid genetic fingerprinting (ribotyping) and pulsed-field gel electrophoresis (PFGE) are emerging as versatile and feasible BST techniques. A phenotypic characterization method, antibiotic resistance analysis, also has the potential to identify the human or animal origin of isolates. However, reference “libraries” of bacterial genetic fingerprints and antibiotic resistance profiles are needed to correctly identify the source of bacteria isolated from environmental water samples.

## **Section A6: Project/Task Description**

### **General Project Description:**

This project has two general objectives: (1) to assess the water quality in Lake Waco and Lake Belton with regard to the relative contributions of fecal bacteria from bovine, human, and other animal contributions to the water bodies and (2) to develop local libraries, genetic and biochemical, that can be used in determining the animal or human nonpoint fecal source contamination of surface water.

### **Water Quality Assessment**

The proposed project will include the review and evaluation of existing data and information pertaining to bacterial contributions and sources to Lake Waco and Lake Belton. New data, of known and specified quality, will be collected and analyzed to differentiate and quantify the relative contributions of bovine livestock and other human and animal bacteria sources into Lake Waco and Lake Belton. This assessment and differentiation between bacteria sources will utilize, and be coordinated with, the development of bacterial source tracking libraries generated by TAMU, EP AREC, and TAMU-CC. This project will provide sufficient documentation of the data and technical analyses conducted that will aid the TFB in communicating the assessment results to watershed stakeholders, the TSSWCB, TCEQ, and the USEPA, as necessary. It is also a goal of this project to obtain support for the technical sufficiency of the project for application in other watersheds from stakeholders, and the TCEQ, TSSWCB, and USEPA Region 6.

### **Sanitary Survey**

A sanitary survey of source regions, as well as information about land use, population density, wastewater and stormwater infrastructure, agricultural practices, and wildlife will provide information to assist in identifying the sources of fecal pollutants within the Lake Waco/Lake Belton watershed study area. This is important for two reasons. First, we must identify the possible sources to ensure that we analyze resident *E. coli* strains from each contributing source. Second, this information will provide the TSSWCB with information not only on the specific animal source of fecal contamination, but also assist in pinpointing the sources geographically.

Parsons will acquire available literature, data, and information that is germane to describing the contributions and define sources of microbial loading in the watersheds. Data analyses will include discussion of temporal (inter-annual, seasonal) and spatial trends in water quality, an evaluation of potential sources, and an identification of data gaps. Special emphasis will be placed on acquiring land use/land cover and human and agricultural census data. This will be a key step in assisting in the planning and execution of the project. Several other types of existing data and information may prove useful in the sanitary survey. These include:

- Reported wastewater permit information, including permit limits, self-reported effluent quality data, violations and inspection reports;

- Hydrologic and meteorological data;
- Land use, population density, and the extent of use of on-site sewerage systems (septic tanks) in the watershed;
- Livestock density and agricultural practices in the watershed from the most recent county-level agricultural census, updated with the most recent dairy cow population estimates from the USDA-NRCS, as well as the abundance and type of confined animal feeding operations;
- Antimicrobial use in feed in the project area, if available;
- Special studies and published reports for the study area.

### **Library Development**

The proposed project will include the development of publicly available ERIC-PCR, RiboPrinter ribotype, PFGE genetic fingerprint and antibiotic resistance profile libraries generated from approximately 1,000 unique *E. coli* isolates from known animal, human and wastewater sources from the Bosque River/Lake Waco and Leon River/Lake Belton watersheds. Approximately 750 water isolates from the above watersheds will be analyzed and compared to the libraries to determine the human or animal sources of bacterial contamination. To make progress towards the development of statewide libraries, approximately 100 *E. coli* isolates from known animal sources in South Texas will also be characterized. A multiphasic approach using standardized methods will be used so that the libraries can be expanded through future Texas §303(d) impaired waterbody studies and data may be exchanged with other researchers.

A critical element of this proposal is the use of the Qualicon RiboPrinter Microbial Characterization System for automated ribotyping of *E. coli* isolates. The RiboPrinter was originally developed for use in identification and BST of microbial isolates for the food industry. There are several advantages of using the RiboPrinter:

- The RiboPrinter is the only automated system for high-throughput genetic typing of bacteria. All bacterial isolate sample processing is automated using standardized reagents and a robotic workstation, providing an exceptional level of reproducibility. Up to 32 samples may be analyzed per day, whereas manual ribotyping methods may require up to several days to complete.
- The system has built-in data analysis of ribotypes, including automated searching of the Qualicon ribotype library and custom identification libraries. This reduces the awkward and subjective interpretation of ribotype fingerprints.
- The electronic ribotype libraries can be shared with other users via a modem, allowing rapid dissemination of data. Furthermore, since the system employs standardized methods and reagents, results obtained from other laboratories using the system are directly comparable.
- Researchers with the United States Geological Survey, Purdue University Calumet, New York Department of Environmental Protection, University of Hawaii and University of

Georgia have recently begun using the RiboPrinter system for BST, creating the possibility of data sharing and development of an expansive ribotype library.

*E. coli* isolates will be selected using an approach to maximize the diversity of strains represented in the library. Livestock and wildlife fecal samples, municipal wastewater, septic system and dairy waste lagoon samples will be obtained from a variety of sources in the Lake Waco and Lake Belton watersheds. Sources will be identified through a sanitary survey to be conducted by Parsons. Parsons will be responsible for collecting and shipping samples to the EP AREC Environmental Microbiology Laboratory for isolation of *E. coli*.

Confirmed *E. coli* bacterial colonies will be screened using a repetitive sequence polymerase chain reaction (ERIC-PCR) method. ERIC-PCR is another 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 fecal, wastewater, etc. 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, therefore a large library of ERIC-PCR fingerprints will be generated. Cultures of selected isolates will be archived and subcultures will be shipped to the other investigators for further analysis.

Following ERIC-PCR analysis, isolates will be ribotyped at the EP AREC using the Qualicon automated RiboPrinter using the restriction enzyme Hind III. This enzyme has been compared to other restriction enzymes by other investigators and found to be useful for BST of *E. coli*. At least two other investigators (Dr. Rose, University of South Florida; Dr. Tseng, Purdue-Calumet) are currently using the RiboPrinter and Hind III for BST of *E. coli*. Both of these investigators have stated they are willing to freely share data, creating the possibility of rapidly expanding the ribotype library.

The isolates will be further characterized using pulsed-field gel electrophoresis (PFGE), another leading method for BST. PFGE is currently being used by the Centers for Disease Control and Prevention (CDC) to track foodborne *E. coli* O157:H7 and *Salmonella* isolates. The isolates will be shipped to TAMU Food and Environmental Microbiology Laboratory (Pillai) for PFGE analysis. The standardized CDC protocol for PFGE analysis of *E. coli* will be followed. CDC currently uses this standardized protocol as the basis of their "PulseNet" outbreak surveillance network which allows public health laboratories nationwide to quickly compare their PFGE fingerprints to the CDC central reference library.

An additional BST technique, antibiotic resistance analysis, will be performed by the TAMU Corpus Christi Environmental Microbiology Laboratory (Mott). This technique follows methods used in the clinical laboratory for evaluating the antibiotic resistance of bacterial isolates. Commonly, the disk diffusion method is used which involves measuring the diameter of zone of inhibition of bacterial growth around a filter disk impregnated with a specific antibiotic. By comparison to resistant and susceptible control strains, the response of the *E. coli* isolates can be

determined. To further standardize and automate the assay, an image analysis system will be used to measure the zones of inhibition and provide electronic archival of data.

By using the combination of ERIC-PCR, RiboPrinter ribotyping, PFGE, and ARA, one of the most comprehensive BST libraries of environmental *E. coli* isolates will be developed. This multiphasic approach will allow the state of Texas to keep pace with developments in BST technology. The validity of the study and conclusions will be strengthened through the use of multiple techniques to identify the sources of contamination. It is also anticipated that approximately 100 blind QC *E. coli* isolates will be analyzed throughout the course of the project to evaluate the robustness of the methodologies and laboratory performance. By using standardized methods, the libraries can be expanded through future projects and the data shared with other BST investigators and regulatory agencies. Peer-reviewed publication of project results is also a goal of the proposed work.

### **Ambient Water Sampling**

As stated previously, one objective of the project is identification and quantification of the relative contributing sources of *E. coli* to six stations in Lake Waco and the Bosque River, and five stations in Lake Belton and the Leon River. The stations are listed in Section B1. Each station will be sampled on ten dates (events). At nine of the sites, five samples will be collected at each event. At the two stations nearest the dam and drinking water intake of each reservoir, ten samples will be collected per event.

Following collection, water samples must be delivered to the City of Waco Laboratory for *E. coli* culturing and enumeration via the membrane filter Modified mTEC method. We will meet a 6 hour holding time for sample delivery to the laboratory.

Following the 24-hour incubation and enumeration, the *E. coli* cultures will be shipped on ice overnight to EP AREC for *E. coli* colony isolation and confirmation, archiving, ERIC-PCR, and ribotyping analysis. *E. coli* cultures will be transferred on ice via overnight courier to TAMU-CC for ARA, and to TAMU for PFGE analysis. A single *E. coli* isolate per sample will be subjected to the ribotyping, ARA, and PFGE analysis, giving a total of 50 isolates typed per station (100 for the two stations near the dams). A total of 650 *E. coli* isolates will be analyzed by ARA, PFGE, and ribotyping.

### **Quantification of Accuracy and Precision in Source Determinations**

This project will provide an estimate of the relative contributions from various fecal sources in the watershed to the observed *E. coli* levels in Lakes Waco and Belton; however, it is important to understand the level of uncertainty that will accompany those estimates. Precision, accuracy, sensitivity, completeness, and representativeness are critical data quality issues affecting uncertainty. Representativeness must be controlled by developing an environmental monitoring program that is characteristic of actual environmental conditions. Accuracy, precision, sensitivity, and completeness can be similarly controlled through careful planning, but also

should be quantified via QC measures. These QC measures include analysis of blank samples, replicate samples, and known standards (in BST, samples of known origin).

BST does not lend itself easily to the same quality control methods as chemical quantification. Blank samples may be irrelevant, and replicate water samples may often yield different *E. coli* strains. We plan to quantify method accuracy and precision through a special QC study with "double-blind" safeguards, as practiced in epidemiological QC:

EP AREC will prepare a list of the more than 1,000 *E. coli* isolates in the known source library for this project, and send this list to the TSSWCB PM. The TSSWCB PM will select 30 isolates from this list from a variety of source categories, and notify EP AREC, TAMU, and TAMU-CC of the 30 isolates to be used in the QC study. EP AREC will then prepare triplicate cultures of the 30 selected *E. coli* isolates in identical slant tubes, each with a removable label indicating their isolate number (but not the source). These 90 tubes will be shipped to the Parsons QAO. The Parsons QAO will randomly select 10 of the 30 isolates (30 of the 90 cultures), replace each label with a new label, numbered from 1 to 30 in random fashion, and record those numbers on a key with the isolate number. The Parsons QAO will then send these 30 slant tubes to Dr. Pillai at TAMU after verifying that there is no way for their source to be identified. The Parsons PM will send the key to the TSSWCB PM. Dr. Pillai will inspect the condition of the cultures, subculture, then ship the cultures to EP AREC. At EP AREC, Dr. Di Giovanni will inspect the condition of the cultures, subculture, then ship the cultures to Dr. Mott at TAMU-CC. The cultures will then be processed through the ribotyping, PFGE, and ARA procedures. Each laboratory will report:

1. Identification of replicate cultures (or groups of isolates which have identical fingerprints) to evaluate precision
2. Identification of cultures to isolate number within the list of 30 isolates to evaluate accuracy of matching to a small subset of isolates
3. Source identification of each unknown based on comparison to all library isolates to evaluate accuracy of source ID based on the total library

Each laboratory will independently send the results to the TSSWCB PM, who will make a copy of the key and results and provide it to EP AREC, TAMU, TAMU-CC, and Parsons. Parsons will evaluate and prepare a brief report on the accuracy and precision of the methods, which will then be reviewed and edited by each of the laboratories.

### **Estimation of *E. coli* Source Contributions**

With ten different sampling events per station, and five samples per station per sampling event, and one isolate typed for each sample, a total of 50 isolates per site will be typed. At the two dam stations, a total of 100 isolates per site will be typed. The number of isolates typed will impact the confidence level around the estimate of the fecal source contributions. Having 50 isolates matched to sources at a given station should permit us to quantify the relative contribution of a

source comprising 20% of the total *E. coli* loading with a precision of approximately plus or minus 11% at the 95% confidence level. Having 100 isolates matched to sources at a station should permit us to quantify the relative contribution of a source comprising 20% of the total *E. coli* loading with a precision of plus or minus 8% at the 95% confidence level.

**Table A.1. Illustration of Predicted Confidence of Source Contribution Estimates**

True Source % Contribution to Fecal Loading	Number of Isolates Ribotyped	Predicted 95% Confidence Interval of Estimated Source Contribution
20%	50	9 – 31%
	100	12 – 28%
30%	50	17 – 43%
	100	21 – 39%
40%	50	26 – 54%
	100	30 – 50%
50%	50	36 – 64%
	100	40 – 60%

**Revisions to the QAPP**

Until the work described is completed, this QAPP shall be revised as necessary and reissued on the anniversary date, or revised and reissued within 120 days of significant changes, whichever is sooner. The last approved version of the QAPP shall remain in effect until revised versions have been approved. If the entire QAPP is current, valid, and accurately reflects the project goals and the organizations policy, the annual re-issuance may be done by a certification that the plan is current, and include a copy of new, signed approval pages for the QAPP.

**Expedited Changes**

Expedited changes to the QAPP may be approved 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 EP AREC or Parsons PM to the TSSWCB PM in writing. They are effective immediately upon approval by the TSSWCB PM and QAO, or their designees. Expedited changes to the QAPP and the reasons for the changes shall be documented, and revised pages shall be initialed by the TWSSCB PM and QAO, EPA Project Officer, and EP AREC or Parsons PM, then distributed to all persons on the QAPP distribution list by the TSSWCB QAO.

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.

**Personnel and Equipment Requirements**

The staff involved in sample collection for library development will be led by a trained, experienced field biologist, with at least a bachelor’s degree in biology or a related discipline and three years of experience in field biology. The staff involved in water sample collection will be led by a person with at least a bachelor’s degree in science or engineering, and at least three

years of experience in environmental data collection in the field. Laboratory staff responsible for analyzing samples for this project will have at least six months of experience in microbiological analysis, and be supervised by personnel with at least a master's degree in microbiology (or the equivalent) and five years of experience in microbiological analysis.

### **Quality Assurance Records**

See [Section A9](#) for a listing of project QA documents and records.

## Section A7: Quality Objectives And Criteria For Measurement Data

The objective of this section is to ensure that data collected meets the data quality objectives (DQOs) of the project. One objective is to identify and quantify the relative contributions of sources of *E. coli* to Lake Waco and Lake Belton, and to quantify the uncertainty of these source contribution estimates. A second objective is to develop libraries, genetic and biochemical, that can be used in determining the animal or human nonpoint fecal source contamination of surface water. Achievement of these objectives will support decisions on how to best target management measures to reduce fecal bacteria levels in Lake Waco and Lake Belton waters. Note that it is not an objective of this project to quantify the ambient levels and loadings of *E. coli* or fecal coliform from these watersheds.

The measurement performance criteria to support the project objective are specified in [Table A.3](#).

**Table A.2. Laboratory Analysis Responsibilities for Water Samples**

<u>Parameter</u>	<u>Laboratory</u>
<i>E. coli</i> culture and enumeration in waters	City of Waco
Total Suspended Solids in water	City of Waco
ARA	TAMU-CC
Ribotyping	EP AREC
ERIC-PCR	EP AREC
PFGE	TAMU

**Table A.3. Data Quality Objectives for Measurement Data**

Parameter	Units	Method Type	Method	Method Description	Storet	MAL	Precision of Laboratory Duplicates*	Accuracy*	Precision of Field Duplicates	Percent Complete
Field Parameters										
Days Since Last Significant Precipitation	Days	Observation	TCEQ SOP	Field observation	72053	NA	NA	NA	NA	90
Flow Severity	1-no flow, 2-low, 3-normal, 4-flood, 5-high, 6-dry	Observation	TCEQ SOP	Field observation	01351	NA	NA	NA	NA	90
Lab Parameters										
Total Suspended Solids (total nonfilterable residue)	mg/L	Non-filterable residue, dried at 105 °C, gravimetric	EPA Method 160.2	gravimetric	00530	4.0	<10: 30% 10-100: 20% >100: 10%	NA	<10: 30% 10-100: 20% >100: 10%	90
<i>E. coli</i> 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
<i>E. coli</i> ribotype	NA	DNA/ image matching	EP AREC SOP	Ribotyping	NA	NA	90% identical	90% correct	75% agreement	90#
<i>E. coli</i> PFGE pattern	NA	DNA/ image matching	CDC SOP	PFGE	NA	NA	90% identical	90% correct	75% agreement	90#
<i>E. coli</i> ERIC-PCR profile	NA	DNA/ image matching	EP AREC SOP	ERIC-PCR	NA	NA	90% identical	90% correct	75% agreement	90#
<i>E. coli</i> 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.

\* accuracy and laboratory method precision will be determined using isolates from known-source samples in a blind procedure, as discussed in Section B5.

## Precision

The precision of laboratory data is a measure of the reproducibility of a result from an analysis repeated. It is strictly defined as a measure of the closeness with which multiple analyses of a given sample agree with each other. Precision is assessed by repeated analyses of a sample. For quantitative microbiological analyses, the method to be used for calculating precision is the one outlined in Standard Methods for the Examination of Water and Wastewater, 20th Edition, section 9020 B.8.b.

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

The  $RPD_{\text{bacteria}}$  should be lower than  $3.27 \Sigma R \log/n$ , where  $R \log$  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 are 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.

## Accuracy

Accuracy is a statistical measurement of correctness and includes components of systemic error. A measurement is considered accurate when the result reported does not differ from the true situation. 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 A.3](#).

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

## Representativeness

Data collected under this project will be considered representative of ambient water quality conditions. Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions typical of a receiving water. The representativeness of the data is

dependent on 1) the sampling locations, 2) the number of samples collected, 3) the number of years and seasons when sampling is performed, 4) the number of depths sampled, and 5) the sampling procedures. Site selection procedures will assure that the measurement data represents the conditions at the site. The goal for meeting total representation of the water body and watershed is tempered by the availability of time and funding. Representativeness will be measured with the completion of sample collection in accordance with the approved QAPP.

### **Comparability**

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

### **Completeness**

The completeness of the data is a measure 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, weather, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 90 percent 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 can not 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. The project team plans to develop a library of approximately 1,000 isolates collected from fecal sources within the Lake Waco and Lake Belton watersheds. This local library will be supplemented by libraries of other investigators. Thus, we believe that sources of a high percentage of the host-specific *E. coli* isolated from this watershed can be identified. It will be a general goal of this project to identify the sources of 70% of the *E. coli* strains isolated from water.

## **Section A8: Special Training Requirements/Certifications**

No special certifications are required, except that personnel involved in use of Global Positioning System (GPS) instruments have been trained in the appropriate use of GPS and have been certified by the TCEQ.

## Section A9: Documentation and Records

Hard copies of general maintenance records, chain of custody forms (COCs), laboratory data entry sheets, calibration logs, and corrective action reports (CARs) will be archived by each laboratory for at least five years. Hard copies of all field data sheets, general maintenance records, chain of custody forms (COCs), field data entry sheets, and corrective action reports will be archived by Parsons for at least five years. In addition, EP AREC will archive electronic forms of all project data for at least five years. A blank CAR form is presented in [Appendix A](#), a blank COC form is presented in [Appendix B](#), and blank field data reporting forms are presented in [Appendix C](#).

The EP AREC laboratory supervisor and Parsons PM will together produce a QA/QC report as part of the quarterly and final reports, which will be kept on file at EP AREC with copies made available upon request. Any items or areas identified as potential problems and any variations or supplements to QAPP procedures noted in the QA/QC 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 water quality monitoring program, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP.

### Special Reporting Formats

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

### Case Narrative

A case narrative is not required for this project.

**Table A.4. Documentation and Records**

Document/Record	Location	Retention	Form
QAPP, Amendments and Appendices	Parsons	5 years	Paper
QAPP Distribution Documentation	Parsons	5 years	Paper
Field Data Sheets	Parsons	5 years	Paper
Chain of Custody Records	Parsons	5 years	Paper
Field SOPs	Parsons	5 years	Paper
Media/Incubation Logs	Labs	5 years	Paper
Laboratory Sample Reception Logs	Labs	5 years	Paper
Laboratory QA Manuals	Labs	5 years	Paper
Laboratory SOPs	Labs	5 years	Paper

Document/Record	Location	Retention	Form
Laboratory Internal/External Standards	Labs	5 years	Paper
Laboratory Instrument Performance	Labs	5 years	Paper
Laboratory Initial Demo of Capability	Labs	5 years	Paper
Laboratory Procedures	Labs	5 years	Paper
Instrument Raw Data Files	Labs	5 years	LIMS Electronic
Instrument Readings/Print Outs	Labs	5 years	Paper
Laboratory Data Reports	Labs	5 years	Paper
Laboratory Data Verification for Integrity, Precision, Accuracy, and Validation	Labs	5 years	Paper
Laboratory Equipment Maintenance Logs	Labs	5 years	Paper
Laboratory Calibration Records	Labs	5 years	LIMS Electronic
Laboratory Corrective Action Documentation	Labs	5 years	Paper
Database Verification	EP AREC/ Parsons	5 years	Electronic
Data QA/QC Verification/Validation	EP AREC/ Parsons	5 years	Paper
QC verification/validation	EP AREC/ Parsons	5 years	Paper
Progress Reports/Final Report/Data	Parsons/ EP AREC/ TSSWCB	5 years	Paper/Electronic

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

## Section B1: Sampling Process Design (Experimental Design)

One goal of this project is to develop localized libraries, genetic and biochemical, that can be used in determining the animal or human fecal source contamination of surface water. The primary sampling design consideration is to obtain as many unique library *E. coli* isolates as possible (with the available resources) from sources representing the diversity and abundance of fecal sources occurring in Texas watersheds. Budget and time constraints will limit the library to a size of approximately 1,000 unique *E. coli* isolates. Potential fecal sources will be identified through a sanitary survey to be conducted by Parsons. Livestock and wildlife fecal samples, municipal wastewater treatment plant effluent, septic system and dairy waste lagoon samples will be obtained from a variety of sources in the Lake Waco and Lake Belton watersheds based on the sanitary survey. Confirmed *E. coli* bacterial colonies will be screened using a repetitive sequence polymerase chain reaction (ERIC-PCR) method. ERIC-PCR is another 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 fecal, wastewater, etc. 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, therefore a library of approximately 1,000 unique ERIC-PCR fingerprints will be generated. Cultures of selected isolates will be archived and subcultures will be shipped to the other investigators for further analysis.

A second goal of this project is to assess the water quality in Lake Waco and Lake Belton with regard to the relative contributions of fecal bacteria from bovine, human, and other animal contributions to the water bodies. One part of this goal is to understand the spatial variation of the source contributions in the lakes and in each of the major tributaries. Thus, sampling sites will be located near the dam and in each arm of both reservoirs, as well as on each of the major tributaries upstream of the reservoirs. Proposed sampling stations are listed in Table B1.

In order to obtain temporally representative results, including wet and dry conditions and seasonal variation, the ambient water sampling will occur on a routine schedule over the course of ten months, and capture dry and runoff-influenced events at their natural frequency, as they occur. There will be no prejudice against rainfall or high flow events, except that the safety of the sampling crew will not be compromised in case of lightning or flooding. If, near the end of the study, the TSSWCB PM and QAO agree that the sampling has not achieved good representativeness of typical conditions, they may restrict the final sampling event(s) to target a particular condition (e.g., rainfall).

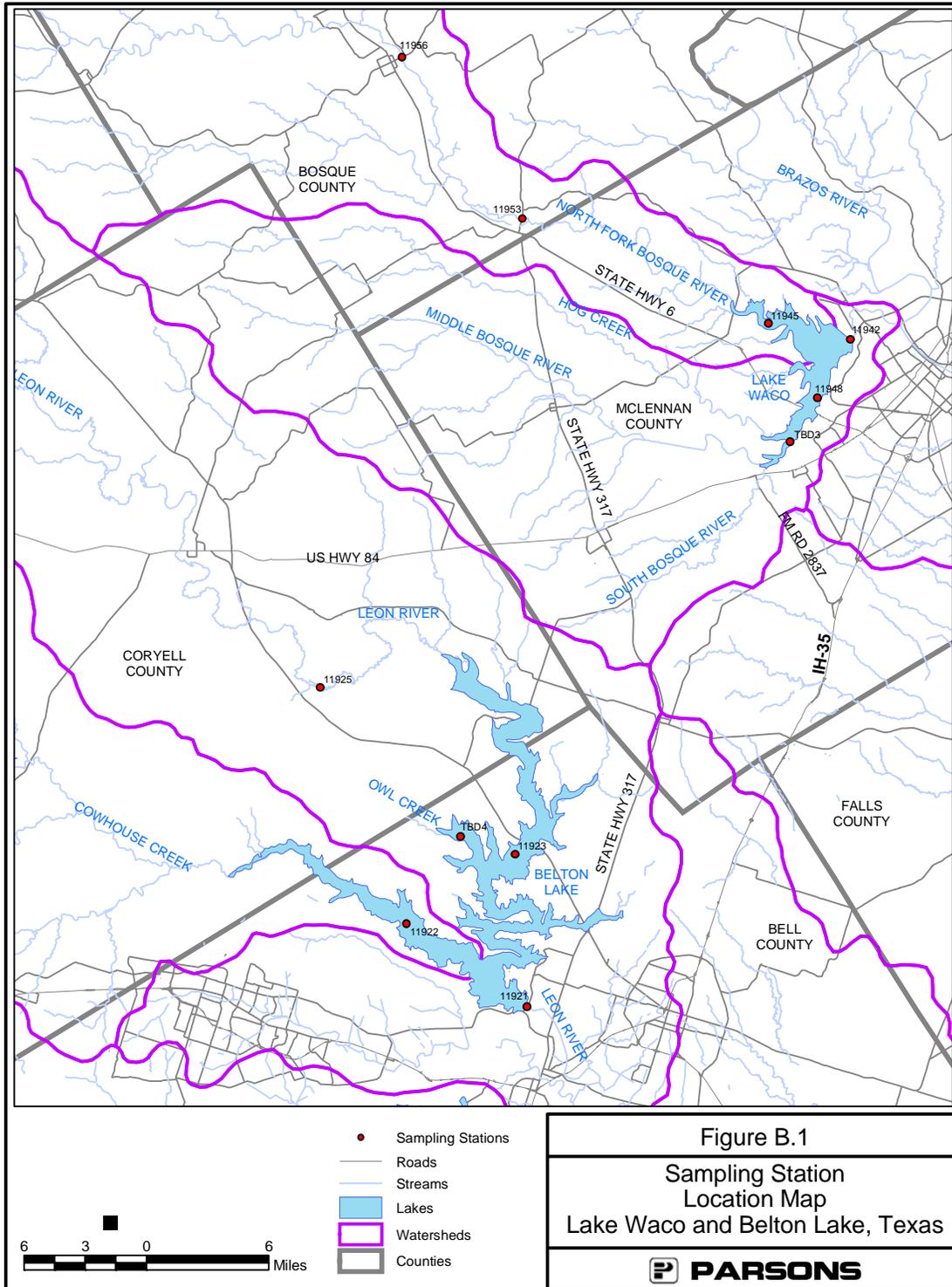
Because *E. coli* populations have been found to vary on fine spatial and temporal scales, we will increase sampling representativeness by collecting five independent water samples per station, 1-2 minutes and 3-10 feet apart, at each event. Typically, this will be done by sampling five points evenly spaced around the perimeter of a boat. At the two deep water stations near the dams and

drinking water intakes of Lake Waco and Lake Belton, the samples will be collected in duplicate.

**Table B1. Proposed Sampling Sites**

<b>Station ID</b>	<b>Station Description</b>	<b>Events</b>	<b>Responsible Agency</b>
11921	Lake Belton near dam	10, monthly	BRA
11922	Lake Belton Cowhouse Creek Arm	10, monthly	BRA
11923	Lake Belton Leon River Arm near headwater	10, monthly	BRA
TBD4	Lake Belton Owl Creek Arm	10, monthly	BRA
11925	Leon River at FM 1829 southeast of North Fork Hood	10, monthly	Parsons
11942	Lake Waco near dam	10, monthly	City of Waco
11945	Lake Waco North Bosque Arm	10, monthly	City of Waco
11948	Lake Waco Middle/South Bosque Arm above SH 6	10, monthly	City of Waco
<del>TBD2</del>	<del>Lake Waco central basin</del>	<del>10, monthly</del>	<del>City of Waco</del>
TBD3	Lake Waco Middle/South Bosque Arm near inlet of Middle/South Bosque River	10, monthly	City of Waco
11953	North Bosque River at SH 56 near Valley Mills	10, monthly	Parsons
<u>11956</u>	<u>North Bosque River at FM 219 northeast of Clifton</u>	<u>10, monthly</u>	<u>Parsons</u>

**Figure B.1 Water Sampling Stations**



J:\742\742880\GIS\watershed\_map1.mxd

## **Section B2: Sampling Method Requirements**

Field sampling personnel will wear clean, disposable, powder-free gloves while collecting all samples.

### **Water Samples**

Typically, water samples will be collected directly from the lake or stream (approximately one foot below the surface) into sterile wide-mouthed polypropylene bottles supplied by the culturing laboratory. 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, and boat access is not practical, staff will use a clean bucket and rope from a bridge 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. Specifically, they must exert care to ensure that the bucket and rope do not come into contact with the bridge. The bucket must be thoroughly rinsed between stations. Buckets are also to be sanitized between sampling stations with a bleach- or isopropyl alcohol-soaked wipe. The first bucketful of water collected from a bridge is used to rinse the bucket 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.

Field observations (flow severity and days since last significant precipitation) are based on SOPs in the TCEQ's Surface Water Quality Monitoring Procedures Manual (1999)

### **Known Source Fecal and Sewage Samples**

To the extent possible, known source samples are collected directly from the source feces. An exception is human samples, which will be collected from septic tanks and septic haulers, sewer lines (lift stations) serving residential areas, and wastewater treatment plants. In some cases, wildlife samples must be collected indirectly, from "found" fecal samples. The sources of these "found" wildlife fecal samples will be identified to the lowest practical taxonomic level by experienced field biologists. These "found" fecal deposits will be photographed. In the case of uncertainty regarding its source, the sample will not be used for library development. No more than ten samples will be collected from the members of the same animal species from a given location, unless those animals do not normally comprise a distinct population of low diversity, but have been assembled temporarily (e.g., a livestock show, animal shelter, or migrating waterfowl). Only a single sample will be collected from an individual animal.

Fresh animal fecal samples will be collected aseptically, using a sterile spatula or swab, into sterile, screw-cap polypropylene specimen tubes, which are then capped and sealed. All sample containers will be labeled with the following information:

- sample type,

- host species,
- collection date,
- collection time,
- sample location,
- and sampler's initials.

All the sample information will be logged into a field log. Samples are immediately placed into an ice chest or refrigerator, and later shipped via overnight courier to the EP AREC laboratory.

**Table B2. Sample Volume, Container Types, Minimum Sample Volume, Preservation Requirements, and Holding Time Requirements.**

Parameter	Matrix	Container	Preservation	Temperature	Sample Volume	Holding Time
Total Suspended Solids	water	Screw-capped polyethylene or glass bottle	none	4°C	100 ml	7 days
<i>E. coli</i>	water	sterile plastic bottle	none	4°C	125 ml	6 hours #
<i>E. coli</i>	Feces, sewage	Sterile screw-capped polypropylene tube	none	4°C	>1 mg	3 days

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

### Documentation of Field Sampling Activities

Field sampling activities are documented on field data reporting forms as presented in [Appendix C](#). For all ambient water sampling, station ID, location, sampling time, date, and depth and sample collector's name/signature are recorded. Detailed observational data are recorded including water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, days since last significant rainfall, estimated hours since rainfall began (if applicable), and flow severity.

### Recording Data

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

- Legible writing with no modifications, write-overs or cross-outs;

- Correction of errors with a single line followed by an initial and date;
- Close-outs on incomplete pages with an initialed and dated diagonal line.

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

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

## Section B3: Sample Handling and Custody Requirements

### Chain-of -Custody

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

### Sample Labeling

Samples are labeled on the container (or on a label attached directly to the container) with an indelible, waterproof marker. Label information includes the site identification, and the date and time of sampling. The COC form will accompany all sets of sample containers.

### Sample Handling

Following collection, samples are placed on ice in an insulated cooler for transport to the laboratory. At the laboratory, samples are placed in a refrigerated cooler dedicated to sample storage. The City of Waco Laboratory Supervisor has the responsibility to ensure that holding times are met with water samples. The holding time is documented on the COC. The EP AREC laboratory has the responsibility to ensure that holding times are met with known source samples. Any problems will be documented with a corrective action report.

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 City of Waco laboratory sample custodian will then enclose the sample COC in the shipping container and send it via overnight courier to the EP AREC laboratory.

### Failures in Chain-of-Custody and Corrective Action

All failures associated with chain-of-custody procedures are immediately reported to the TSSWCB PM. These include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The TSSWCB PM, in consultation with the TWSSCB QAO will determine if the procedural violation may have

compromised the validity of the resulting data. Any failures that have potential to compromise data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB in the monthly progress report. Corrective action reports will be maintained by the TWWSCB PM.

## **Section B4: Analytical Methods Requirements**

### **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  $35\pm 0.5^{\circ}\text{C}$  for two hours to resuscitate stressed bacteria, then incubated at  $44.5\pm 0.2^{\circ}\text{C}$  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- $\beta$ -D-glucuronide, which is catabolized to glucuronic acid and a red- or magenta-colored compound by *E. coli* that produce the enzyme  $\beta$ -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 Isolation and Purification**

*E. coli* in water samples will be isolated and enumerated by the City of Waco laboratory using modified mTEC agar, Modified EPA Method 1103.1 (EPA/821/R-97/004, March 2000, *Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Escherichia coli*). 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.

### **EC Analysis**

Confirmed *E. coli* bacterial colonies will be screened using a repetitive sequence polymerase chain reaction (ERIC-PCR) method. ERIC-PCR is another 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 fecal, wastewater, etc. 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, therefore a large library of ERIC-PCR fingerprints will be generated. Cultures of selected isolates will be archived in tryptone soy broth (TSB) with 20% glycerol at  $-70^{\circ}\text{C}$  in cryovials and subcultures will be shipped to the other investigators for further analysis.

Following ERIC-PCR analysis, isolates will be ribotyped at the EP AREC 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 TAMU laboratory, and by ARA at the TAMU-CC Laboratory.

The analytical methods are listed in [Table A.3](#) of [Section A7](#). No EPA-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 E, F, G, and H.

### **Failures in Analytical Methods Requirements and Corrective Action**

In the event of a failure in the analytical system, the TSSWCB PM and QAO will be notified. The laboratory supervisor, in consultation with the TSSWCB QAO and PM, will decide to accept or reject data associated with the analysis, based on best professional judgment. They may determine if the existing sample integrity is intact, if the analysis can or should be repeated, and if re-sampling should be done. The resolution of the situation will be reported to the TSSWCB in the quarterly report.

## Section B5: Quality Control Requirements

Table A.3 in Section A7 lists the required accuracy, 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 than 20% of the lowest value of the batch.

### Field Blanks

Field blanks consist of sterile distilled water that is taken to the field and transferred to the appropriate container in precisely the same manner as a sample during the course of a sampling event. They are used to assess the contamination from field sources such as air borne materials, carryover from prior sampling sites, and containers. The analysis of field blanks should yield values less than the MAL. When target analyte concentrations are high, blank values should be less than 20% of the lowest value of the batch. Field blanks will be collected at a frequency of 5% or greater for TSS and *E. coli*. For *E. coli*, blanks will be processed through *E. coli* culturing and quantification steps, but not processed by ERIC-PCR, ribotyping, PFGE, or ARA.

### Field duplicates

Field duplicates utilized in this study will be split replicates, collected by splitting a composite sample, immediately after compositing in the field, into two bottles. Field duplicate samples are sealed, handled, stored, shipped, and analyzed in the same manner as the primary sample. Field duplicates for TSS will be collected at a frequency of 1 in 20 (5%). Because only a small subset of the bacterial colonies cultured from a water sample are typed, it is expected that two samples will yield slightly different results, depending on which colonies are selected for typing. Thus, duplicate results from a single sampling event are not a meaningful measure of quality; they are expected to disagree. However, the overall source identification, based on multiple sampling events and many isolates, should be consistent from duplicate samples. If this difference is large, it may indicate problems with sampling, analysis, experimental design, or typing. In this project, field duplicate samples will be collected for each of the sampling events at the sampling stations at the dams of Lake Waco and Lake Belton. It will be a goal of this project that there is at least 75% agreement between the sources identified, as illustrated in the example below:

Isolate #	Duplicate	1 Duplicate	2 Same?
1	Cattle	Cattle	Yes
2	Cattle	Cattle	Yes
3	Cattle	Cattle	Yes
4	Cattle	Human	No
5	Cattle	Dog	No
6	Dog	Dog	Yes
7	Dog	Dog	Yes
8	Dog	Dog	Yes
9	Horse	Human	No
10	Human	Human	Yes
11	Human	Human	Yes
12	Human	Human	Yes
			9/12 = 75%

**Method Accuracy and Precision Study**

BST does not lend itself easily to the same QC methods as chemical quantification because each measurement is qualitative, not quantitative. Blank samples are less relevant, and replicate water samples may often yield different *E. coli* strains. Laboratory method accuracy and precision will be quantified through a special QC study with “double-blind” safeguards, as practiced in epidemiological QC. EP AREC will prepare a list of the more than 1,000 *E. coli* isolates in the known source library for this project, and send this list to the TSSWCB PM. The TSSWCB PM will select 30 isolates from this list from a variety of source categories, and notify EP AREC, TAMU, and TAMU-CC of the 30 isolates to be used in the QC study. EP AREC will then prepare triplicate cultures of the 30 selected *E. coli* isolates in identical slant tubes, each with a removable label indicating their isolate number (but not the source). These 90 tubes will be shipped to the Parsons QAO. The Parsons QAO will randomly select 10 of the 30 isolates (30 of the 90 cultures), replace each label with a new label, numbered from 1 to 30 in random fashion, and record those numbers on a key with the isolate number. The Parsons QAO will then send these 30 slant tubes to Dr. Pillai at TAMU after verifying that there is no way for their source to be identified. The Parsons PM will send the key to the TSSWCB PM. Dr. Pillai will inspect the condition of the cultures, subculture, then ship the cultures to EP AREC. At EP AREC, Dr. Di Giovanni will inspect the condition of the cultures, subculture, then ship the cultures to Dr. Mott at TAMU-CC. The cultures will then be processed through the ribotyping, PFGE, and ARA procedures. Each laboratory will report:

4. Identification of replicate cultures (or groups of isolates which have identical fingerprints) to evaluate precision
5. Identification of cultures to isolate number within the list of 30 isolates to evaluate accuracy of matching to a small subset of isolates

6. Source identification of each unknown based on comparison to all library isolates to evaluate accuracy of source ID based on the total library

Each laboratory will independently send the results to the TSSWCB PM, who will make a copy of the key and results and provide it to EP AREC, TAMU, TAMU-CC, and Parsons. Parsons will evaluate and prepare a brief report on the accuracy and precision of the methods, which will then be reviewed and edited by each of the laboratories.

The objective for precision in this study is that the source indicated by each of the three replicates of each *E. coli* strain is identical, for 9 of the 10 *E. coli* strains (90%) or more. The objective for accuracy in this study is that the true source of unknown samples are identified correctly by each method (ERIC-PCR, ribotyping, ARA, and PFGE) in 27 of the 30 cases (90%) or more.

Each laboratory method includes additional required QC measures, such as verification of *E. coli* colonies. Detailed laboratory QC requirements are contained within each individual laboratory method.

#### **Failures in Quality Control and Corrective Action**

Sampling QC excursions are evaluated by the Parsons PM, in consultation with the TSSWCB QAO. In that differences in field duplicate sample results are used to assess the entire sampling process, including environmental variability, the arbitrary rejection of results based on predetermined limits is not practical. Therefore, the professional judgment of the Parsons PM and TSSWCB QAO will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility. Field blank values exceeding the acceptability criteria may automatically invalidate the sample, especially in cases where high blank values may be indicative of contamination. Notations of blank contamination are noted in the quarterly report and the final QA/QC Report.

Corrective action will involve identification of the possible cause of the contamination failure where possible. Response actions will typically include re-analysis of questionable samples. In some cases, a site may have to be re-sampled to achieve project goals.

Laboratory measurement QC failures are evaluated by the laboratory supervisor, in consultation with the EP AREC PM and the TSSWCB QAO. The disposition of such failures and conveyance to the TSSWCB PM are discussed in Section B4 under Failures or Deviations in Analytical Methods Requirements and Corrective Actions.

## Section B6: Equipment Testing, Inspection, & Maintenance Requirements

The equipment requiring periodic testing, inspection, and maintenance is listed in Table B.3. All laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in Standard Methods for the Examination of Water and Wastewater, 20<sup>th</sup> Edition, Section 9020 (APHA, 1998). Records of all tests, inspections, and maintenance will be maintained when in use and log sheets kept showing time, date, recorded temperature and analyst signature. These records will be available for inspection by the TSSWCB.

To minimize downtime of all measurement systems, backup equipment or common spare parts will be made available if any piece of equipment fails during use so that repairs or replacement can be made quickly, allowing measurement tasks to be resumed.

**Table B.3. Equipment Inspection and Maintenance Requirements**

<b>Equipment</b>	<b>Relevant Testing, Inspection and Maintenance Requirement</b>
Thermometers	SM 9020B 3.a
Balances	SM 9020B 3.b
pH meter	SM 9020B 3.c
Water deionization units	SM 9020B 3.d
Media dispensing apparatus	SM 9020B 3.f
Autoclaves	SM 9020B 3.h
Refrigerator	SM 9020B 3.i
Freezer	SM 9020B 3.j
Membrane filter equipment	SM 9020B 3.k
Ultraviolet sterilization lamps	SM 9020B 3.l
Biological safety cabinet	SM 9020B 3.m
Water baths	SM 9020B 3.n
Incubators	SM 9020B 3.o
Microscopes	SM 9020B 3.p
Glassware and plasticware	SM 9020B 4.a
Utensils and containers	SM 9020B 4.b
Dilution water bottles	SM 9020B 4.c
Reagent-grade water quality	SM 9020B 4.d
Use test for reagent water, media, membranes	SM 9020B 4.e
Qualicon Riboprinter	manufacturer's instructions
PCR thermal cycler	manufacturer's instructions
Gel electrophoresis apparatus	manufacturer's instructions
Centrifuge	manufacturer's instructions

## Section B7: Instrument Calibration and Frequency

There are no field instruments or equipment requiring calibration. The instruments requiring calibration are listed in Table B.4. 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 B.4. Instrument Calibration Requirements**

<b>Equipment</b>	<b>Relevant Calibration Requirement</b>
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:

TAMU-CC and EP AREC – balances are calibrated using approved weights by laboratory personnel.

## **Section B8: Inspection/Acceptance Requirements for Supplies and Consumables**

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

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

All required data to be used for this project will be collected in accordance with this QAPP.

## **Section B10: Data Management**

Data for this project will be produced at the City of Waco Laboratory, EP AREC, TAMU, and TAMU-CC, as well as field observations at each of the field monitoring stations.

### **Field Observations**

Field observations will be recorded on field data reporting forms ([Appendix C](#)). These forms will be reviewed for accuracy and copied by the person(s) performing the water sampling, then provided to the Parsons PM along with a copy of the sample COC form, who will review them for accuracy and completeness, and store them in the project files in Parsons' Austin, Texas office.

All COC and field observations data are manually entered into an electronic database by Parsons personnel under supervision of the Parsons PM. Field data and COC information in the database will be verified by field personnel and/or a data analyst. The electronic database will be created in Microsoft Access software on an IBM-compatible microcomputer with the Windows 2000 Operating System. The project database will be maintained on a Windows NT network drive, which is backed up to tape media every night.

Original data recorded on paper files are stored for at least five years in a locked, restricted-access, fire-resistant storage area in the Parsons' Austin office. Electronic data files on the Parsons network are archived to CD-ROM after approximately one year, then maintained in the above storage area.

### **City of Waco Laboratory Data**

All field samples will be logged upon receipt, COC's will be checked for number of samples, proper and exact I.D. number, signatures, dates, and type of analysis specified. TSSWCB will be notified if any discrepancies are found and laboratory analysis will not occur until proper corrections are made. All samples will be stored at 4°C until analysis. Bacteriological samples will be given a unique identification number and logged into the Laboratory Information Management System (LIMS). Enumerated bacteriological data will be manually entered into the LIMS system for electronic storage. The server housing the LIMS is backed up on magnetic tape daily and retained for one to two weeks. At least 10% of all data manually entered in the LIMS database will be reviewed for accuracy to ensure that there are not any transcription errors. The lab will provide EP AREC and Parsons PM with electronic copy of data on a quarterly basis. Hard copies of data will be printed and housed in the laboratory for a period of five years. All other COC and bacteriological records related to QA/QC of bacteriological procedures will be housed at the City of Waco Water Quality Laboratory for five years. Chemical analysis records related to the Bacterial Source Tracking study that are collected by the City of Waco will be kept on-site for a period of ten years.

### **TAMU Laboratory Data**

Data collection will begin upon receipt of new *E. coli* isolates from EP AREC. Unique identification numbers provided by EP AREC for each isolate will be recorded in handwritten notebooks as well as in the chain of custody log. Each isolate's growth pattern on LB+MUG will be recorded in hand written notebooks and electronic format at the end of every week. The molecular analysis of these isolates will be recorded both as a digital image and as a printed image in the research personnel's notebook. All digital images 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 the EP AREC and Parsons PMs with a draft electronic copy of the data on at least a quarterly basis.

### **EP AREC 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 all PMs, TFB, TSSWCB, and EPA a draft electronic copy of the data on at least a quarterly basis.

### **TAMU-CC Laboratory Data**

All *E. coli* isolate deliveries will be logged upon receipt of samples, COC's will be checked for proper shipping, number of samples sent, proper and exact I.D. number sent, signatures, dates, and type of analysis specified. TSSWCB will be notified if any discrepancies are found and laboratory analysis will not occur until proper corrections are made. All samples will be stored at 4°C until analysis. Instantaneous reading and Interpretation with NCCLS M100, EXPERT and QC, Test results, Infection Control Reports and selected plate images will be stored in the Windows-based BIOMIC database. Backups on CD or equivalent will be made after each analysis. The lab will provide EP AREC and Parsons PM with electronic copy of data on a quarterly basis.

### **Data Validation**

Using the review of laboratory data, data that are not representative of environmental conditions because they were generated through poor field or laboratory practices will not be submitted to the TSSWCB. This determination will be made by the laboratory supervisors, Parsons PM, TSSWCB QAO, and other personnel having direct experience with the data collection effort, as specified in [Table D.1](#). This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the following checks specified in [Table D.1](#).

## **Data Reporting**

Data will be reported according to the standards of the TSSWCB. A data review checklist ([Appendix D](#)) will assist in ensuring that the reported data are reported correctly.

## **Data Dissemination**

At the conclusion of the project, the Parsons and EP AREC PMs will provide a copy of the complete project electronic database via recordable CD-ROM media to the TSSWCB PM, along with the final report. The TSSWCB may elect to take possession of all project records, or Parsons and EP AREC will maintain them according to the Project Records retention schedule in [Table A.4](#). Summaries of the data will be presented in the final project report. The TSSWCB may disseminate the validated data and report.

## Section C1: Assessments and Response Actions

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

**Table C.1. Assessments and Response Actions**

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	EP AREC and TFB 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	EP AREC and TFB project managers will report to TSSWCB project manager in monthly 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	EP AREC and TFB PMs have 30 days to respond in writing to the TSSWCB QAO to address corrective actions

### Corrective Action

The EP AREC and TFB Project Managers are responsible for implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the TSSWCB QAO.

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

## **Section C2: Reports to Management**

### **Laboratory Data Reports**

Laboratory data reports contain the results of all specified QC measures listed in [Section B5](#), including but not limited to bottle and equipment blanks, field blanks, and field duplicates. This information is reviewed by the EP AREC and Parsons PMs and compared to the pre-specified acceptance criteria to determine acceptability of data. This information is available for inspection by the TSSWCB.

### **Reports to EP AREC Project Management**

The TAMU and TAMU-CC laboratory supervisors will provide routine verbal or written communications to the EP AREC PM. At a minimum, they will provide data and a verbal or written progress report to support the progress reports and meetings with the TSSWCB. In addition, these laboratories will provide electronic and/or hard copies of all analytical and bacteria tests results. Furthermore, these laboratories will assist in the preparation of reports to the TSSWCB evaluating bacteria strains and sources. Parsons will also provide the EP AREC with the results of the special QC study.

### **Reports to TFB Project Management**

The Parsons, BRA, and City of Waco PMs will provide routine verbal or written communications to the TFB PM. At a minimum, they will provide data and a verbal or written progress report to support the progress reports and meetings with the TSSWCB. In addition, the City of Waco laboratory will provide electronic and/or hard copies of all bacteria tests results. Furthermore, Parsons will assist in the preparation of reports to the TSSWCB evaluating bacteria sources. Parsons will also provide the TFB with the results of the special QC study.

### **Reports to TSSWCB Project Management**

Quarterly Progress Reports - summarizes activities for each task; reports problems, delays, and corrective actions; and outlines the status of each task's deliverables. Submitted by the TFB and EP AREC PMs.

### **Monitoring Systems Review Checklist and Report of Significant Corrective Actions**

Following the annual audits, the monitoring systems audit checklist along with recommendations and corrective actions is sent to the TSSWCB by the TFB and EP AREC PMs.

## **Section D1: Data Review, Validation and Verification**

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

The procedures for verification and validation of data are described in [Section D2](#), below. The Parsons, BRA, and City of Waco PMs are responsible for ensuring that field data collected by their staff are properly reviewed, verified, and submitted in the required format for the project database. Likewise, the laboratory supervisors are responsible for ensuring that laboratory data are reviewed, verified, and submitted in the required format for the project database. The TSSWCB QAO is responsible for validating that all data collected meet the data quality objectives of the project.

## Section D2: Validation and Verification Methods

All data will be verified to ensure they are representative of the samples analyzed and locations where measurements were made, and that the data and associated QC data conform to project specifications. The staff and management of the EP AREC laboratory, the TAMU laboratory, the TAMU-CC laboratory, the City of Waco and its laboratory, the BRA, and Parsons are responsible for the integrity, validation and verification of the data each field and laboratory task generates or handles throughout each process. The field and laboratory QA tasks ensure the verification of field data, electronically generated data, and data on chain-of-custody forms and hard copy output from instruments.

Verification, validation and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified (listed by task in [Table D.1](#)) are evaluated against project specifications ([Section A7](#) and [Section B5](#)) and are checked to ensure the verification of raw data for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data, or identified using computer-based statistical software. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues that can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TSSWCB QAO to establish the appropriate course of action, or the data associated with the issue are rejected. The performance of these tasks is documented by completion of the data review checklist ([Appendix D](#)).

The Parsons and EP AREC PMs and TSSWCB QAO are each responsible for validating that the verified data are scientifically valid, defensible, of known precision, accuracy, integrity, meet the data quality objectives of the project, and are reportable to the TSSWCB.

**Table D.1. Data Review, Verification, and Validation Procedures**

<b>Data to be Verified</b>	<b>Field Supervisor and Staff</b>	<b>Laboratory Supervisor and Staff</b>	<b>PM/QAO Task *</b>
Collection and analysis techniques consistent with SOPs and QAPP	X	X	
Field QC samples collected for all analytes as prescribed in the QAPP	X		X
Field documentation complete	X		X
Instrument calibration data complete		X	X
Bacteriological records complete	X	X	X
Sample documentation complete	X	X	X
Field QC results within acceptance limits	X		X
Sample identifications	X	X	X
Chain of custody complete/acceptable	X	X	X
Sample preservation and handling	X	X	
Holding times	X	X	X
Instrument calibration data		X	X
QC samples analyzed at required frequencies	X	X	X
QC samples within acceptance limits	X	X	X
Internal/external standards		X	X
Instrument readings/printouts		X	X
Calculations	X	X	X
MALs for lab analyses		X	X
Laboratory data verification for integrity, precision, accuracy and validation		X	X
Laboratory data reports		X	X
Data entered in required format	X	X	X
Site ID number assigned			X
Valid STORET codes			X
Absence of transcription error	X	X	X
Reasonableness of data	X	X	X
Electronic submittal errors	X	X	X
Sampling and analytical data gaps	X	X	X

\* TSSWCB Project Manager and QAO will monitor only 10% of data for QA/QC purpose.  
 All other entities are required to inspect 100% of the data prior to approval

### Section D3: Reconciliation with Data Quality Objectives

Data that have been reviewed, verified, and validated will be summarized at each station individually, as well as all stations together, for their ability to meet the data quality objectives of the project and the informational needs of water quality agency decision-makers. General questions that will be asked, and the metrics on which they will be evaluated, are listed in [Table D.2](#).

**Table D.2. Methods for Reconciling Results with Data Quality Objectives**

<b>Evaluation Issue</b>	<b>Specific Measures</b>
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 fraction of <i>E. coli</i> 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

**Appendix A**  
**Corrective Action Report**  
**CAR #:** \_\_\_\_\_

Date: \_\_\_\_\_

Area/Location: \_\_\_\_\_

Reported by: \_\_\_\_\_

Activity: \_\_\_\_\_

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

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Possible causes:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Recommended Corrective Actions:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

CAR routed to: \_\_\_\_\_

Received by: \_\_\_\_\_

Corrective Actions taken:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Has problem been corrected?:

YES

NO

Immediate Supervisor: \_\_\_\_\_

Project Manager: \_\_\_\_\_

Quality Assurance Officer: \_\_\_\_\_

**Appendix B**  
**Chain of Custody Record**

**CHAIN OF CUSTODY RECORD**  
**PARSONS**  
 8000 CENTRE PARK DRIVE, SUITE 200  
 AUSTIN, TEXAS 78754

Project: Assessment of Bacterial Sources Impacting Lakes Waco and Belton							Remarks:					
Name and signature of collector:							Air bill #					
Station ID	Sample ID	Media Code	Sample Type	Preservation	Collection Date:	Time:	Num containers	Analyses			Tag ID	Remarks
								EC	TSS	Other		
Relinquished by: (Signature):					Date:	Time:	Received for Waco lab by:			Date:	Time:	Laboratory Notes:
Relinquished by: (Signature):					Date:	Time:	Received for EP AREC lab:			Date:	Time:	
Project Manager (Signature):												

<b>Station ID</b>	<b>Station Description</b>	<b>Media Codes</b>	
11921	Lake Belton near dam	SW	surface water
11922	Lake Belton Cowhouse Creek Arm	FM	fecal matter
11923	Lake Belton Leon River Arm near headwater	SD	sediment
TBD4	Lake Belton Owl Creek Arm	MW	municipal wastewater effluent
11925	Leon River at FM 1829 southeast of North Fork Hood	IW	industrial wastewater effluent
11942	Lake Waco near dam	WW	other wastewater
11945	Lake Waco North Bosque Arm	DS	domestic sewage (raw)
11948	Lake Waco Middle/South Bosque Arm above SH 6	SF	surface soil
<del>TBD2</del>	<del>Lake Waco central</del>	PW	potable water
TBD3	Lake Waco Middle/South Bosque Arm near inlet of Middle/South Bosque River	OT	other
11953	North Bosque River at SH 56 near Valley Mills	AS	animal sewage
<u>11956</u>	<u>North Bosque River at FM 219 NE of Clifton</u>	WP	wipe or swab sample

**Sample Types**

A	Ambient
FD	field duplicate
FL	field blank
EB	equipment blank
BK	other blank

---

**Appendix C**  
**Field Data Reporting Form**



**Appendix D  
Data Review Checklist**

**Field Data Review**

- A. QC samples collected for all analytes as prescribed in the QAPP? \_\_\_\_\_
- B. Are duplicate and blank results acceptable? \_\_\_\_\_
- C. Are field QC results attached to this review? \_\_\_\_\_
- D. Field documentation includes the following:
  - (1) Identification of individual(s) collecting sample(s)? \_\_\_\_\_
  - (2) Sample ID number and site location? \_\_\_\_\_
  - (3) Sample collection date, depth, and time? \_\_\_\_\_
  - (4) Site observations (i.e. weather, unusual flow, etc)? \_\_\_\_\_
  - (5) Unusual occurrences that may affect water quality? \_\_\_\_\_
  - (6) Sample collection problems? \_\_\_\_\_

**Data Format and Structure**

- A. Are there any duplicate *Sample Id* numbers? \_\_\_\_\_
- B. Are the Sample prefixes correct? \_\_\_\_\_
- C. Are all *Sample Id* numbers 7 characters? \_\_\_\_\_
- D. Are station ID numbers assigned? \_\_\_\_\_
- E. Are sampling *Dates* in the correct format, DD/MM/YYYY? \_\_\_\_\_
- F. Is the sample *Depth* substantially greater or less than 0.3 meters or 1 foot? \_\_\_\_\_
- G. Is the *Comment* field filled in where appropriate? \_\_\_\_\_
- J. Values represented by a valid parameter (*STORET*) code with the correct units? \_\_\_\_\_
- K. Are there any duplicate measurements for the same *ID* and *STORET*? \_\_\_\_\_
- L. Are there any invalid symbols in the Greater Than/Less Than (*GT/LT*) field? \_\_\_\_\_
- N. Is the sampling *Time* based on the 24 hour clock (e.g. 13:04)? \_\_\_\_\_

**Data Quality Review**

- A. Holding times confirmed? \_\_\_\_\_
- B. MALs consistent with those in the QAPP? \_\_\_\_\_
- C. Outliers confirmed and documented? \_\_\_\_\_
- D. Documentation (verified error log) provided? \_\_\_\_\_
- E. Checks on correctness of analysis or data reasonableness performed? \_\_\_\_\_
- F. Have at least 10% of the data in the database been reviewed against the data sheets? \_\_\_\_\_
- G. Chain of custody record properly filled out and available for review? \_\_\_\_\_

Y = Yes    X = No    N/A = Not applicable

**Data Review Checklist (page 2 of 2)**

Explain any answers that may indicate a problem with the data (attach another page if necessary):

Date Submitted: \_\_\_\_\_

TAG Series: \_\_\_\_\_

Date Range: \_\_\_\_\_

Data Source: \_\_\_\_\_

Comments (attach README.TXT file if applicable):

**Signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Appendix E****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

<b>MASTER MIX</b>	<b>Amt (uL)</b>	<b>Final Calc</b>	<b>Final Units</b>
dH <sub>2</sub> O	<b>31.5</b>		
10X PCR buffer I w Mg	<b>5</b>	1	X
20 mM dNTP	<b>0.5</b>	200	uM each
ERIC Primer Mix	<b>5</b>	600	nM each
BSA (30 mg/ml)	<b>2.5</b>	1.5	ug/uL
AmpliTaQGold (Units)	<b>0.5</b>	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  $\mu$ l ethidium bromide each additional use

14. Mix 10  $\mu$ l 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  $\mu$ l of ERIC-PCR Blank into the first two lanes
- b. Load 10  $\mu$ l of 100 bp ladder (0.5  $\mu$ g) into third lane on gel
- c. Load 10  $\mu$ l of PCR reactions into next 8 lanes
- d. Load 10  $\mu$ l of 100 bp ladder (0.5  $\mu$ g) into lane 12
- e. Load 10  $\mu$ l of PCR reactions into next 8 lanes
- f. Load 10  $\mu$ l of 100 bp ladder (0.5  $\mu$ g) into lane 21
- g. Load 10  $\mu$ l of PCR reactions into next 6 lanes
- h. Load 10  $\mu$ l of 100 bp ladder (0.5  $\mu$ g) into lane 28
- i. Load 10  $\mu$ l 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 F**

### **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**

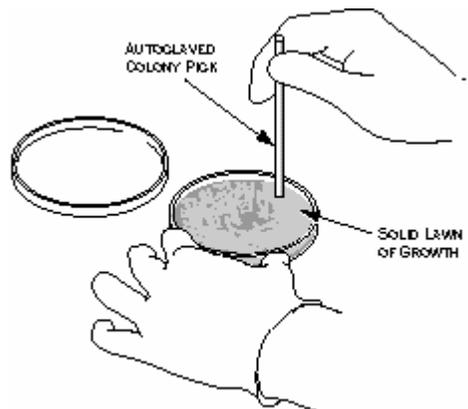
- a) Locate the 250 mL twist-top bottle of sample buffer supplied in Pack # 1 Install the twist cap.
- b) Transfer about 5 mL of buffer to a sterilized disposable 15 mL intermediate working tube.

### 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  $\mu$ L of sample buffer from the intermediate tube.  
For Gram positive samples (e.g. *S. aureus* and *L. innocua* QC strains), add 40  $\mu$ L 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  $\mu$ L of sample buffer.

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

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

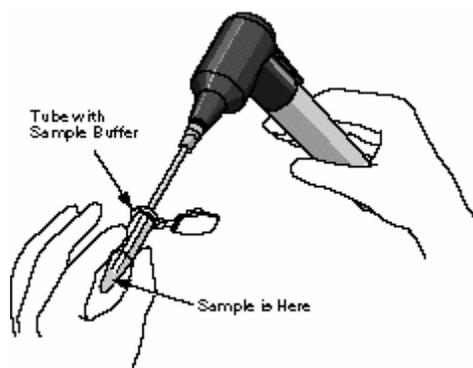
## 5. Mix the Samples

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

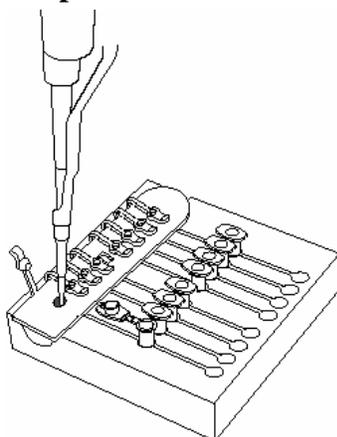
1. Making certain not to touch the sample end of the pick, place the pick into one of the filled sample tubes.
2. While holding the tube with the open end facing away from you, carefully attach the pick to the hand-held mixer. The fit of the pick in the coupling will be loose.

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

3. Press the ON lever on the mixer for about 5 seconds.
4. Release the lever and carefully remove the colony pick. The sample liquid should appear turbid.
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.
6. Cap the sample tube.
7. Move the tube to the top row of the sample preparation rack. This indicates that the tube is filled.



## 6. Transfer the Samples to the Sample Carrier



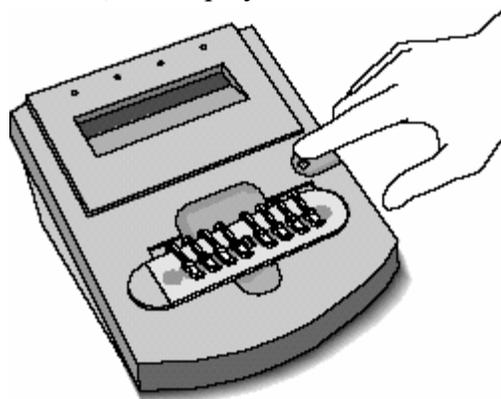
1. Open the lid covering the first well of the sample carrier.
2. Using a 100  $\mu$ L pipetter, pipette 30  $\mu$ L of sample from the microcentrifuge tube into the well.
3. Close the lid cover for the well.
4. Repeat for remaining samples using a new pipette tip for each sample.

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

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

## **Creating and Loading a Batch**

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

- *EcoRI* batches (VCA)
- *PstI* batches (VCB)
- *PvuII* batches (VCC)

You can also create special batches:

- Restriction Enzyme Flexibility batches
- **Substitute Enzyme batches (including *Hind III*)**

From the Instrument Control Base Window:

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

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

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

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

7. For each well in the sample carrier, choose the type (Sample or Control [QC Number]) from the Sample Type field. The system defaults to Sample.
  8. Once you define the Sample Type as Sample, type in the name you actually want to use. This information will appear as Sample Label in the Data Analysis software screens.
  9. You can change the RiboGroup library name if needed. Do this by clicking on the button next to the field with the mouse button. A pop up menu appears listing your choices. If you want to add a new library name, move the pointer to the line and click with the mouse button to get a cursor, then type in the new library name. Once you have saved this file, the new name will be added to the pop up list for future use. Do **NOT** change the DuPont ID field. If you select one of the QC strains, the system automatically enters QC in the DuPont ID and RiboGroup Library fields. Do not change these names. If you wish, you may enter a name for the Custom ID library.
10. Repeat for the other seven samples.
  11. Click on Save and Submit Batch to Instrument.

## Loading Disposables

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

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

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

### 1. Check the DNA Preparation Waste Container

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

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

**WARNING!** Do not unscrew the cap from the DNA Preparation waste container if the fluid level has risen into the cap. First pour the excess waste liquid into the liquid biohazard waste.

**WARNING!** When replacing container make sure that the cap is properly threaded in place. If the cap is only partially threaded, it can snag the pipette during operation.

## 2. Load the Sample Carrier

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

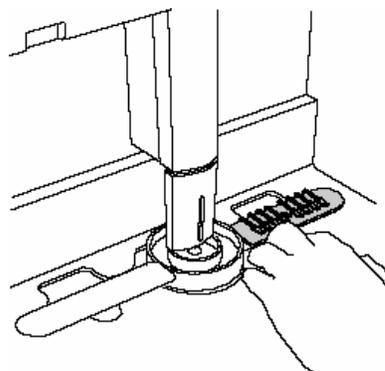
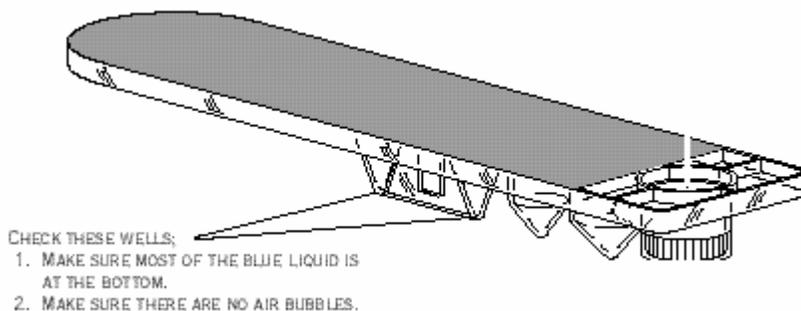
**CAUTION!** Place the rounded edge of the sample carrier on your right as you view the characterization unit. Position the carrier this way to insure correct identification of the sample wells.

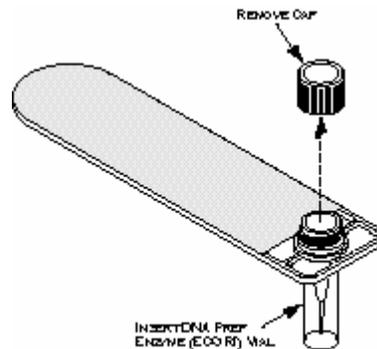
## 3. Load the DNA Prep Carrier

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

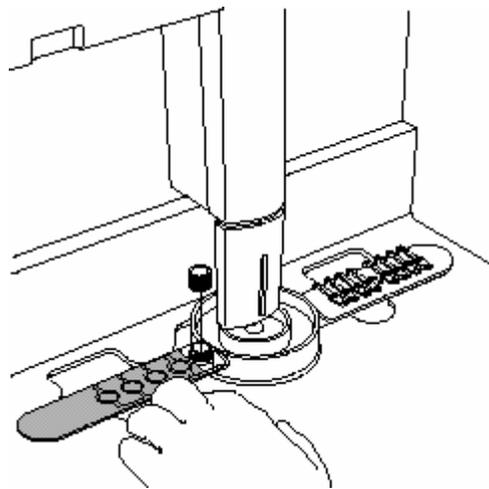
50 U/ $\mu$ L: 26.5  $\mu$ L *Hind* III and 26.5  $\mu$ L of NEB 10X Buffer 2

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





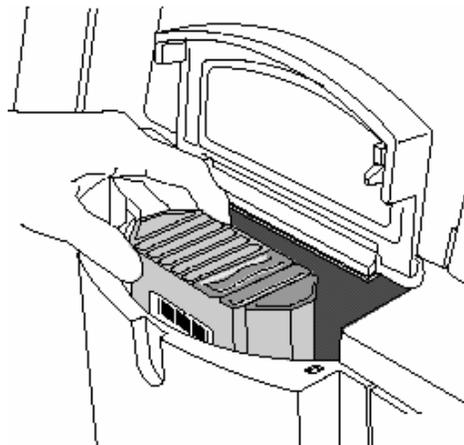
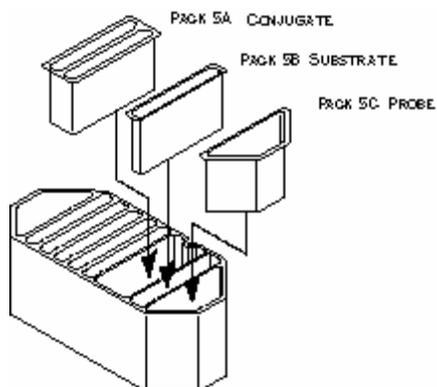
5. Remove the cap from the Enzyme vial.
6. Insert the vial into the carrier.
7. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.
8. Push the DNA Prep carrier down firmly until it snaps into place.



#### 4. Load the MP Base and Carousel

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

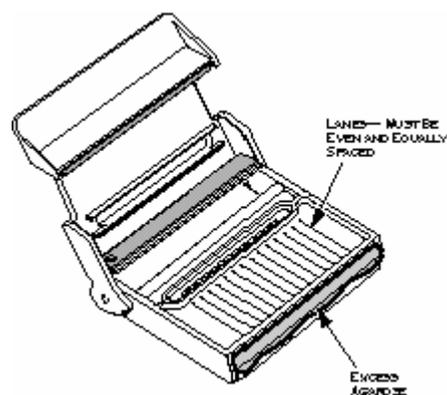
3. Remove each insert from its pouch. Tap the powdered reagent packs gently to bring all powder to the bottom of the packs. Place reagent packs in the MP base and load the base in the carousel.

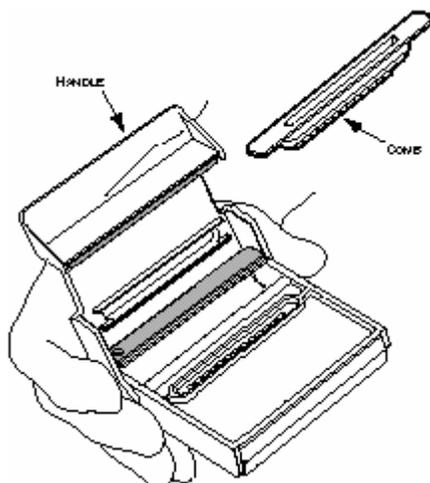


*CAUTION! Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.*

### 5. Load the Gel Cassette

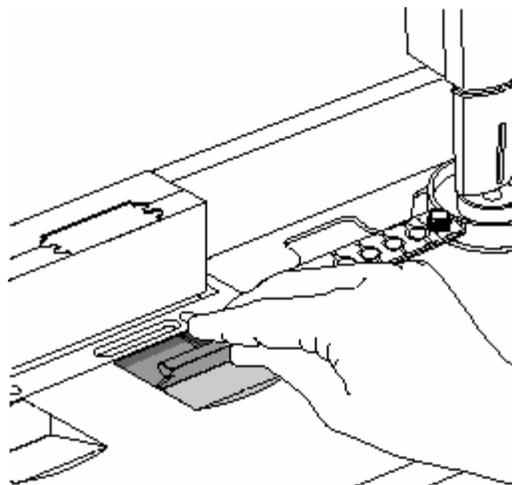
1. Remove the gel cassette from its package.
2. Grasp one end of the rubber comb and gently pull the comb from the cassette.
3. Unfold the handle of the cassette towards you until the handle snaps into place.
4. Check the front edge of the gel cassette and the lanes of the gel.





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

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.

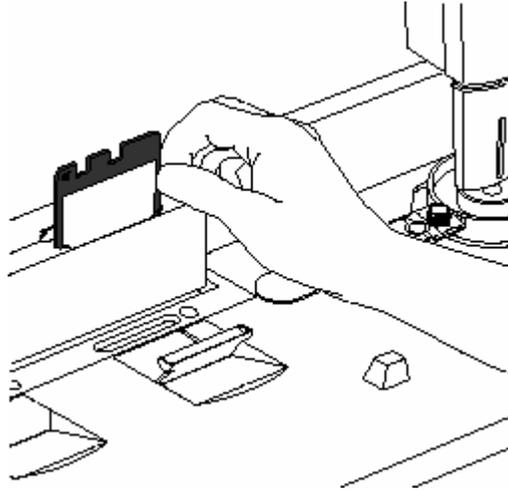


6. Press the cassette forward firmly until it snaps into place.

## 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**

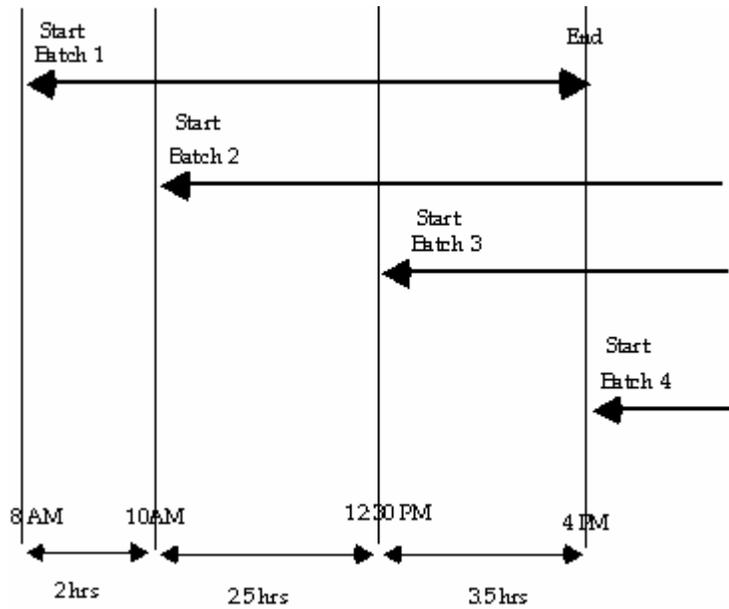
The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. [Other batches](#) may take longer to process.

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 G**  
**Laboratory Protocol for Molecular Subtyping of *Escherichia coli* by Pulsed Field Gel Electrophoresis (PFGE)**

**Day 0**

Streak overnight broth culture of TSB onto TSA for confluent growth using inoculating loop. Incubate cultures at 37° C for 14-18 hrs.

**Day 1**

1. Prepare 1% SeaKem Gold:1% SDS agarose in TE Buffer (10 mM Tris:1 mM EDTA, pH-8.0). Melt agarose in microwave and store in 55° C water bath until use.
2. Transfer 2 ml of Cell Suspension Buffer (CSB) (100 mM Tris: 100 mM EDTA, pH-8.0) to tubes. Use sterile cotton swab that has been moistened with CSB to remove confluent growth from agar plates; suspend cells in CSB by spinning swab gently to disperse cells.
3. Adjust concentration of cell suspension to Optical Density value of 1.35 at 610 nm wavelength of light using a spectrophotometer. Concentration of cells can be changed by adding cells or CSB as needed.
4. Transfer 0.4 ml adjusted cell suspension to labeled 1.5-ml microcentrifuge tubes at room temperature.
5. Add 20 µl of Proteinase K (20 mg/ml stock) to each tube and mix gently with pipette tip.
6. Add 0.4 ml melted 1% SeaKem Gold:1% SDS agarose to the 0.4 ml cell suspension; mix by gently pipetting mixture up and down a few times. Maintain temperature of melted agarose by keeping flask in 55° C water bath.
7. Immediately, dispense part of mixture into appropriate wells of plug mold. Make two plugs per specimen. Allow plugs to solidify at room temperature for 10 min.
8. Prepare Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl). Calculate the total volume of Cell Lysis/Proteinase K Buffer needed as follows:
  1. 5 ml Cell Lysis Buffer per tube + 25 µl Proteinase K stock solution (20 mg/ml) per tube.
  2. Measure correct volumes into appropriate size test tube or flask and mix well.
9. Add 5 ml of Proteinase K/Cell Lysis Buffer to each tube for cell lysis of plugs.

10. Transfer plugs from plug molds into tubes containing Proteinase K/Cell Lysis Buffer. Place tubes into rack in 54° C shaker water bath for 1.5 hrs.
11. Pre-heat sterile reagent grade water to 50° C so that plugs can be washed two times with 10 ml of water.
12. Remove tubes from water bath and pour off lysis buffer into discard container.
13. Add 10 ml of sterile pre-heated water to each tube and shake tubes in 50° C water bath for 10 min.
14. Pour off water from the plugs and repeat wash steps once more. Pre-heat TE Buffer (10 mM Tris:1 mM EDTA, pH-8.0) in 50° C water bath so that the plugs can be washed four times with 10 ml of buffer.
15. Pour off water and add 10 ml pre-heated TE buffer and shake vigorously in 50° C water bath for 10 min.
16. Pour off TE and repeat wash steps three more times.
17. Decant last wash step and add 5 ml TE. Store plugs at 4° C until restriction digest.

## **Day 2**

1. Dilute 10X H buffer (Roche Molecular Biochemicals) 1:10 with sterile reagent grade water.
2. Add 200 µl diluted H buffer to labeled microcentrifuge tube. Carefully remove plugs from TE with spatula and place in sterile disposable Petri dish.
3. Cut 2.0 mm slice from plug with scalpel and transfer to tubes with diluted H buffer. Replace remaining plug into TE buffer and store at 4° C
4. Incubate sample and control plug slices in 37° C water bath for 5 min.
5. After incubation, remove buffer from plug slice using a micropipettor. Dilute 10X H buffer 1:10 and add *Xba*I restriction enzyme (50U/sample). Mix thoroughly.
6. Add 200 µl restriction enzyme mixture to each tube. Close tube and mix by tapping gently.
7. Incubate sample and control plug slices in 37° C water bath for 1.5 hrs.

8. Cast agarose gel by preparing 1% SeaKem Gold agarose in 0.5X TBE buffer. Melt agarose completely and place in 50° C water bath until use.
9. Carefully pour agarose into level gel form fitted with comb.
10. Place black gel frame in electrophoresis chamber. Add 2.2 L freshly prepared 0.5X TBE.
11. Turn on cooling unit (14° C) and pump (set at 70 for 1 liter/min flow rate).
12. Remove restricted plug slices from 37° C water bath. Remove enzyme/buffer mixture and add 200 µl 0.5X TBE. Incubate at room temperature for 5 min.
13. Remove comb after gel solidifies for 30 min.
14. Remove restricted plug slices from tubes and load into appropriate wells with spatula. Make sure there are no air bubbles.
15. Cover plugs and fill wells with remaining melted 1% SeaKem Gold agarose. Allow to harden for 5 min. Unscrew end gates from form; remove excess agarose from sides and bottom of stand with a Kimwipe. Carefully place gel inside black gel frame in electrophoresis chamber. Close cover of unit.
16. Run electrophoresis user program 1 in unit.

### **Day 3**

1. When electrophoresis is over, turn off equipment and stain gel with Ethidium Bromide (0.5 µg/ml) made in 400 ml 0.5X TBE. Stain for 30 min with agitation.
2. Pour off Ethidium Bromide solution and place gel in 400 ml of 0.5X TBE to destain for 15 min. After 15 min. pour off TBE and add fresh 0.5X TBE and continue destaining for 15 min.
3. Place gel on UV light box and photograph using Kodak CDC and software. Save digital photograph and print hardcopy for notebook.

**Appendix H**  
**Laboratory Protocol for Antibiotic Resistance Analysis by the Kirby Bauer**  
**Disk Diffusion Method**

The Kirby Bauer Disk Diffusion Method is described in the following document from the NCCLS: *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-2nd edition (2002). M31-A2 Vol.22 No.6*. A copy of this method is available upon request.

The selection of antibiotics for this study will be based on frequency of usage in humans or animals in the study area, representation of the major classes of antimicrobials active against gram negative organisms, and use in previous studies of resistance in *E. coli*. Where possible, at least one representative of the major classes of antimicrobial substances will be included. In some instances, only one drug will be tested as representative of all the drugs in its class, per NCCLS. In these cases, organisms resistant to the representative drug have routinely shown resistance to the other drugs in the same class (e.g.: tetracycline).

**Appendix I**  
**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 J**

### **Laboratory Protocol for Isolation and Confirmation of *Escherichia coli* from Fecal Specimens**

*Note: All collection and handling of fecal specimens should be performed using protective gear (i.e. latex or nitrile gloves). Specimens should be handled aseptically to ensure sample quality and minimize exposure of personnel to pathogens.*

1. Fecal specimens should be refrigerated as soon as possible after collection and shipped in insulated coolers with ice packs sufficient to keep the specimens between 1–4°C. Ship by next day courier to:

Dr. George D. Di Giovanni  
Texas A&M Ag. Research and Ext. Ctr.  
1380 A&M Circle  
El Paso, TX 79927  
915-859-9111

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

2. Using a bacteriological loop, streak a loopful of fecal material onto a labeled modified mTEC agar plate (EPA/821/R-97/004, Modified EPA Method 1103.1; <http://www.epa.gov/nerlcwww/RecManv.pdf>) for isolation of *E. coli* colonies.
3. Invert the plate and incubate at 35±0.5°C for 2 h.
4. After the 2-h incubation at 35±0.5°C, incubate the plate inverted at 44.5±0.2°C for 20–24 h.
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.
9. At the discretion of the laboratory, additional biochemical tests such as urease, indole and citrate tests may be performed.

## Appendix K

### Laboratory Protocol for Isolation and Confirmation 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  $44.5 \pm 0.2^\circ\text{C}$  incubation, the plates should be immediately stored at  $4^\circ\text{C}$  until shipment to prevent growth of non-*E. coli* coliforms on the plates.
3. Plates with red or magenta colored colonies should be parafilm 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  $1-4^\circ\text{C}$  and ship by next day courier to:

Dr. George D. Di Giovanni  
Texas A&M Ag. Research and Ext. Ctr.  
1380 A&M Circle  
El Paso, TX 79927  
915-859-9111

5. Presumptive *E. coli* from the Modified mTEC plates will be isolated and confirmed as described in the protocol for fecal specimens.