Texas Nonpoint Source Grant Program

Watershed protection plan development for the Navasota River below Lake Limestone

TSSWCB Project # 15-50

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

Revision #0

prepared by

Texas AgriLife Research
Texas Water Resources Institute

and the

Texas A&M University Department of Soil and Crop Sciences

Effective Period: upon signature through September 30, 2016

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

Lucas Gregory
TWRI Quality Assurance Officer
241A Centeq
2260 TAMU
College Station, Texas 77843-2260
lfgregory@ag.tamu.edu

This Page Left Blank Intentionally

Section A1: Approval Sheet

Quality Assurance Project Plan (QAPP) for the *Watershed protection plan development for the Navasota River below Lake Limestone* project.

Texas State Soil and Water Conservation Board (TSSWCB)

Name: Ashley Wendt
Title: TSSWCB Project Manager (PM)

Signature:_____ Date:_____

Name: Mitch Conine
Title: TSSWCB Quality Assurance Officer (QAO)

Signature:_____ Date:_____

Texas AgriLife Research – Texas Water Resources Institute (TWRI)

Name: Kevin Wagner
Title: Project Leader

Signature:_____ Date:_____

Name: Lucas Gregory
Title: TWRI QAO

Signature:_____ Date:_____

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

Name: Terry Gentry
Title: Associate Professor & Project Co-Lead, Soil and Aquatic Microbiology Laboratory (SAML) Director

Signature:_____ Date:_____

Name: Jacqueline Aitkenhead-Peterson
Title: Associate Professor & Nutrient and Water Analysis (NAWA) Lab Director

Signature:_____Date:_____

Section A2: Table of Contents

Section: Title

List of Acronyms and Abbreviations

AWRL	ambient water reporting limits
BMP	best management practice
BST	bacterial source tracking
CAR	corrective action report
COC	chain of custody
CRP	Clean Rivers Program
CWA	Clean Water Act
DMRG	data management reference guide
DQO	data quality objectives
FDC	flow duration curve
GIS	geographic information system
IT	information technology
LCSD	laboratory control sample duplicate
LDC	load duration curve
LOQ	limit of quantification
LULC	landuse/landcover
mTEC	modified membrane Thermotolerant *E. coli*
NAWA	Nutrient and Water Analysis Laboratory
NLCD	national land cover data set
NPS	nonpoint source
NRCS	Natural Resource Conservation Service
OSSF	on-site sewage facility
PM	Project Manager
QA	quality assurance
QAM	quality assurance manual
QAO	Quality Assurance Officer
QAPP	quality assurance project plan
QC	quality control
RPD	relative percent difference
RUAA	recreational use attainability analysis
SAML	Soil and Aquatic Microbiology Laboratory
SCSC	Department of Soil and Crop Sciences
SOP	standard operating procedure
SWCD	Soil and Water Conservation District
SWQMIS	surface water quality monitoring information system
TCEQ	Texas Commission on Environmental Quality
TMDL	total maximum daily load
TNI	The NELAC Institute
TPDES	total pollution discharge elimination system
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas AgriLife Research, Texas Water Resources Institute
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WPP	watershed protection plan
WQMP	water quality management plan

Section A3: Distribution List

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

Texas State Soil and Water Conservation Board

PO Box 658
Temple, TX 76503

Name: Ashley Wendt
Title: TSSWCB PM

Name: Mitch Conine
Title: TSSWCB QAO

Texas AgriLife Research - Texas Water Resources Institute

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

Name: Kevin Wagner
Title: TWRI Associate Director & Project Lead

Name: Lucas Gregory
Title: TWRI QAO

Texas AgriLife Extension Service - Department of Soil and Crop Sciences

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

Name: Terry Gentry
Title: Associate Professor & Project Co-Leader

Name: Jacqueline Aitkenhead-Peterson
Title: Associate Professor

Section A4: Project/Task Organization

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

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

Ashley Wendt, TSSWCB PM

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

Mitch Conine; TSSWCB QAO

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

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

Kevin Wagner, Associate Director, TWRI; Project Lead

The TWRI Project Lead is responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract and in the project QAPP; assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB PM.

Lucas Gregory, QAO, Field Supervisor and Data Manager

Responsible for determining that the QAPP meets the requirements for planning, QA and QC. Conducts audits of field and laboratory systems and procedures. Responsible for maintaining the official, approved QAPP, as well as conducting quality assurance audits in conjunction with TSSWCB personnel.

Responsible for acquisition, verification, and transfer of data to the TSSWCB PM. Oversees data management for the project. Performs data quality assurances prior to transfer of data to the Texas Commission on Environmental Quality (TCEQ) in the

format specified in the most recent version of the Surface Water Quality Monitoring (SWQM) Data Management Reference Guide (DMRG). Ensures that the data review checklist is completed and data is submitted with appropriate codes. Provides the point of contact for the TSSWCB PM to resolve issues related to the data and assumes responsibility for the correction of any data errors.

Responsible for supervising all aspects of the sampling and measurement of surface waters and other field parameters. Responsible for the collection of water samples and field data measurements in a timely manner that meet the quality objectives specified in Section A7 (Table A7.1), as well as the requirements of Sections B1 through B8. Responsible for field scheduling, staffing, and ensuring that staff is appropriately trained. Reports status, problems, and progress to TWRI PM.

SCSC – Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas. Responsible for bacterial source tracking (BST) and nutrient analysis.

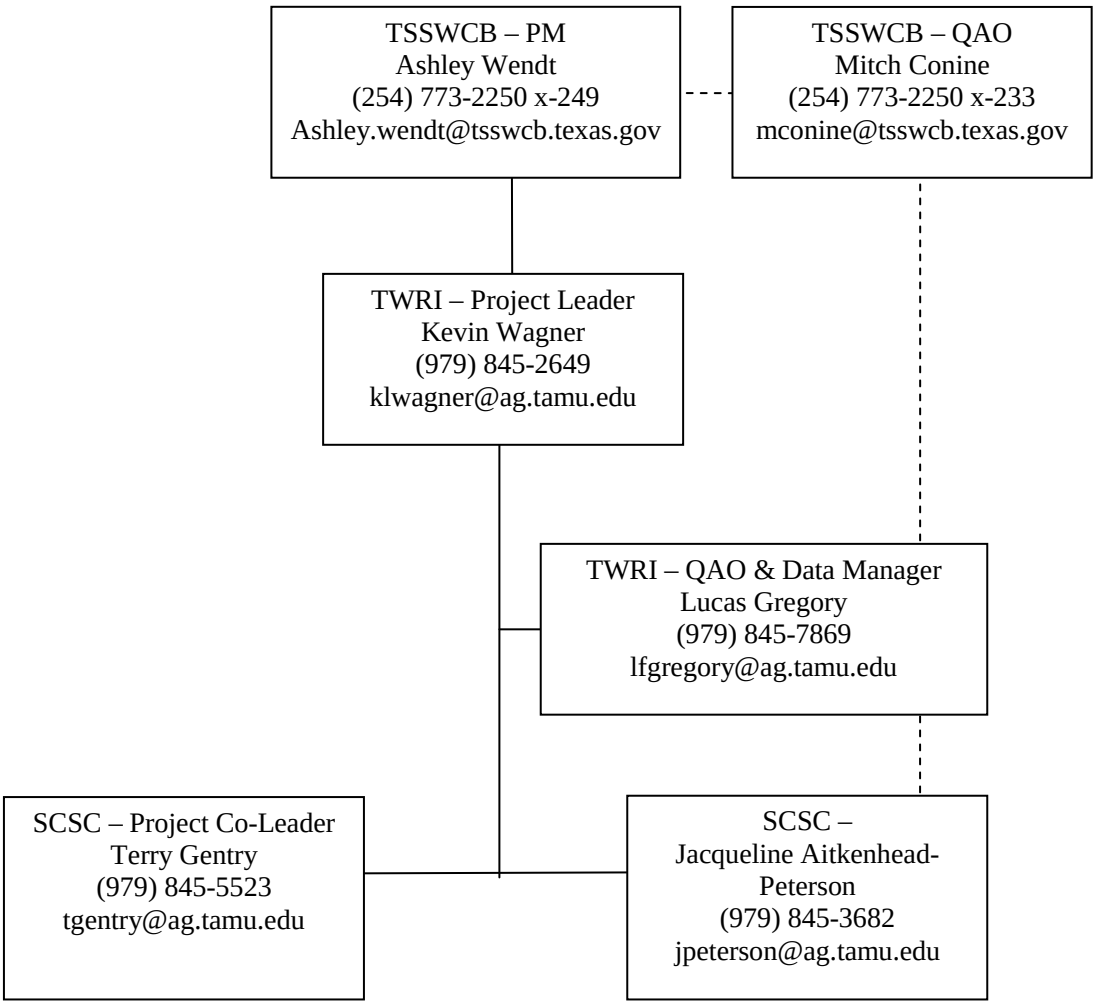
Terry Gentry, Associate Professor, SCSC; SAML Director; Project Co-Lead

Responsible for performing BST analysis and related activities. This includes ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and its requirements specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems.

Jacqueline Aitkenhead-Peterson, Associate Professor, SCSC; NAWA Director

Responsible for performing nutrient analysis and related activities. This includes ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and its requirements specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with the approved QAPP and identify potential problems.

Figure A.4-1. Project Organization Chart



Section A5: Problem Definition/Background

The Navasota River is a part of the larger Brazos River basin and rises in McClennan County. The river is impounded in several locations with Lake Limestone in Freestone, Leon and Limestone Counties being the largest. At the outfall of the Sterling C. Robertson Dam downstream, the Navasota River Below Lake Limestone (Segment 1209) flows south for approximately 126 miles where it joins the Brazos River west of the town of Navasota. While flowing along the boundaries of Brazos, Grimes, Leon, Madison and Robertson counties, the river traverses some of the few remaining bottomland hardwood habitat in the state. According to the *2012 Texas Integrated Report and 303(d) List*, this segment of the river was noted to have elevated levels of *E. coli* that do not support the state's primary contact recreation water quality standard as early as 2002 and still remain elevated according to recently evaluated data. Further this report, suggests potential sources of pollution contributing to the *E. coli* include nonpoint sources from municipal runoff, on-site sewage facilities, municipal point source discharges and other non-point sources.

The Navasota River below Lake Limestone watershed encompasses parts of Brazos, Freestone, Grimes, Leon, Limestone and Robertson counties in east-central Texas. The watershed is predominantly rural and encompasses portions of the Northern Blackland Prairie, Southern Post Oak Savanna, San Antonio Prairie, and Flood Plains and Low Terraces as described in the U.S. Environmental Protection Agency's (USEPA) Level IV Ecoregions of Texas. Specifically, land covers in the watershed are predominantly mixed forests and managed pastures or rangelands. Limited amounts of cropland and urban areas also exist with the cities of Bryan and College Station being the largest by far. With the diverse land uses across the watershed, the development of a watershed protection plan that addresses pollutant loadings from multiple sources is the most appropriate mechanism to restore water quality in the river.

As the Navasota River Below Lake Limestone is a predominantly rural watershed, a Recreational Use Attainability Analysis (RUAA) was initiated on the waterbody in late 2009 to assess the current level of use and historic uses of the waterbody to determine if the currently applied primary contact recreation standard is appropriate. According to information presented by TCEQ at the 2012 Brazos River Basin Clean Rivers Program meeting (available online at: http://www.brazos.org/Basin%20Highlights/2013-Status-RUAAs-Basin.pdf), the Navasota River Below Lake Limestone will not be recommended for a standards change from the current primary contact recreation standard. This effectively means that the waterbody will again be designated as a 5c waterbody on the next iteration of the 303(d) List. Additionally, RUAAs are in progress on tributaries of the Navasota River Below Lake Limestone including Country Club Branch (1209D), Wickson Creek (1209E), Cedar Creek (1209G), Duck Creek (1209H), Gibbons Creek (1209I), Shepherd Creek (1209J) and Steele Creek (1209K). Each of these waterbodies is also listed as impaired for elevated levels of bacteria. The results of these RUAAs are not yet available; however, available evidence suggests that several of these waterbodies will not be recommended for a standards change thus cementing their place on the 303(d) List until other measures can be taken. With this recommendation of the Navasota River Below Lake Limestone and the potential

recommendation of at least some of its tributaries to remain designated for primary contact recreation use, the development of a plan to restore water quality to meet its designated standards is appropriate. Information gleaned on the use, physical and hydrologic characteristics, and features of the waterbody and documented in the *Central and Southeast Texas Recreational Use Attainability Analyses Project: Navasota River Below Lake Limestone (Segment 1209) Comprehensive RUAA* will be incorporated into the watershed protection plan (WPP) development process as appropriate. (http://www.tceq.texas.gov/assets/public/waterquality/standards/NavasotabelowLimestoneCompRUAAFinalReport.pdf)

A supplemental water quality monitoring project that is implementing a portion of the *Implementation Plan for Three TMDLs for Indicator Bacteria in the Carters Creek Watershed* (http://www.tceq.texas.gov/assets/public/waterquality/tmdl/85carters/85A-CartersCreekIPlan-Approved.pdf) will also provide useful information on pollutant loading from the largest urbanized area in the watershed and allow updated loading calculations for this subwatershed to be developed.

Further, a substantiated understanding of the sources contributing to the overall *E. coli* loading to the waterbody are needed to enable appropriate plans to be developed. While that are many potential sources contributing *E. coli* within the waterbody, the relative contribution of their loading is not well understood. Establishing a better understanding of the respective loadings from each source will enable the development of a more effective restoration plan.

Section A6: Project Goals and Task Description

To address water quality impairments and concerns in the Navasota River below Lake Limestone, as described in the *2012 Texas Integrated Report* of Surface Water Quality for Clean Water Act Sections 305(b) and 303(d) and identified by stakeholders, the Texas Water Resources Institute will initiate a watershed planning process to develop strategies to effectively restore the waterbody to meet state requirements and local stakeholder needs. This project will utilize portions of the "Three-Tier Approach for Bacteria TMDL Development" as recommended in the Bacteria TMDL Task Force Report submitted to TCEQ and TSSWCB. Tier 1 and Tier 2 recommended tasks will be combined to develop a better understanding of the hydrology, water quality, potential causes and sources for the impairment and will cultivate stakeholder ideas to include in the development of a WPP for the Navasota River Below Lake Limestone.

Local participation will be a cornerstone of this planning process as it will ultimately be up to these same entities, groups and individuals to implement the WPP once completed and approved. TWRI will facilitate the development of an organized stakeholder group by working with landowners, public officials, special interest groups and agencies that have been identified as potential participants. Members of this group will be asked to provide guidance on pollutant sources assessments, establishment of water quality goals, and selection of management strategies during the development of the WPP. Routine stakeholder meetings and meetings with local soil and water conservation districts (SWCDs) will be held to ensure the continued engagement of stakeholders in the planning process. Additionally, a project website will be developed and hosted to serve as an informational resource for the watershed.

Data gathering and subsequent assessments of this data will also support the development of the WPP. TWRI will gather and utilize existing data and prior studies to identify water quality issues, characterize the watershed, identify potential sources of pollution, evaluate current loadings, establish needed loading reductions, and prioritize critical areas for implementation. Load duration curves (LDCs) will be utilized to determine needed loading reductions at critical points in the watershed, while the development of a watershed geographic information system (GIS) supplemented with stakeholder feedback will allow critical areas within the watershed needing specific management prescriptions to be identified systematically.

Supplemental water quality monitoring will also be conducted. TWRI will conduct bi-weekly sampling and streamflow monitoring at a key index site for the waterbody (TCEQ Station 11785) for one year. Duplicate samples will be collected with one set being delivered to SAML at Texas A&M for *E. coli* enumeration using EPA 1603 method and the other being delivered to NAWA lab for non-regulatory nutrient analysis including nitrate, nitrite, ammonium, and orthophosphorous. Monitoring this site, which is in the downstream impaired portion of the river, will provide much needed *E. coli* counts and stream flow data that will improve LDC loading and loading reduction estimates. BST will also be conducted on water samples collected at this site. SAML will perform both library-dependent and library independent methods to each of the water samples collected through this project. In total, 96 *E. coli* isolates will be screened with the enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR) / RiboPrinting (RP) library dependent method and each sample will be process to determine the

presence or absence of Bacteroidales BST markers using library independent methods. Collectively, this will allow for a more detailed understanding of the temporal variability in water quality as well as the specific sources of bacterial loading to the river; all of which will be conveyed to watershed stakeholders to enhance their understanding of the watershed and facilitate informed WPP development.

Using information gleaned through this project, TWRI will work with stakeholders to develop a WPP that satisfies USEPA's 9 key elements of watershed based plans. In short, the developed WPP will clearly define pollutant sources and estimated loadings, will establish management recommendations and estimate their pollutant loading reductions. Additionally, the plan will also describe technical and financial assistance needs, an education plan, a project schedule with interim measurable milestones, indicators to measure progress and a long-term monitoring plan.

The purpose of this QAPP is to clearly delineate the QA policy, management structure, and procedures, which will be used to implement the QA requirements necessary to acquire existing data, develop LDCs, conduct water quality monitoring and complete BST analysis under tasks 3 and 4. Table A6-1 provides specific subtask milestones for this project.

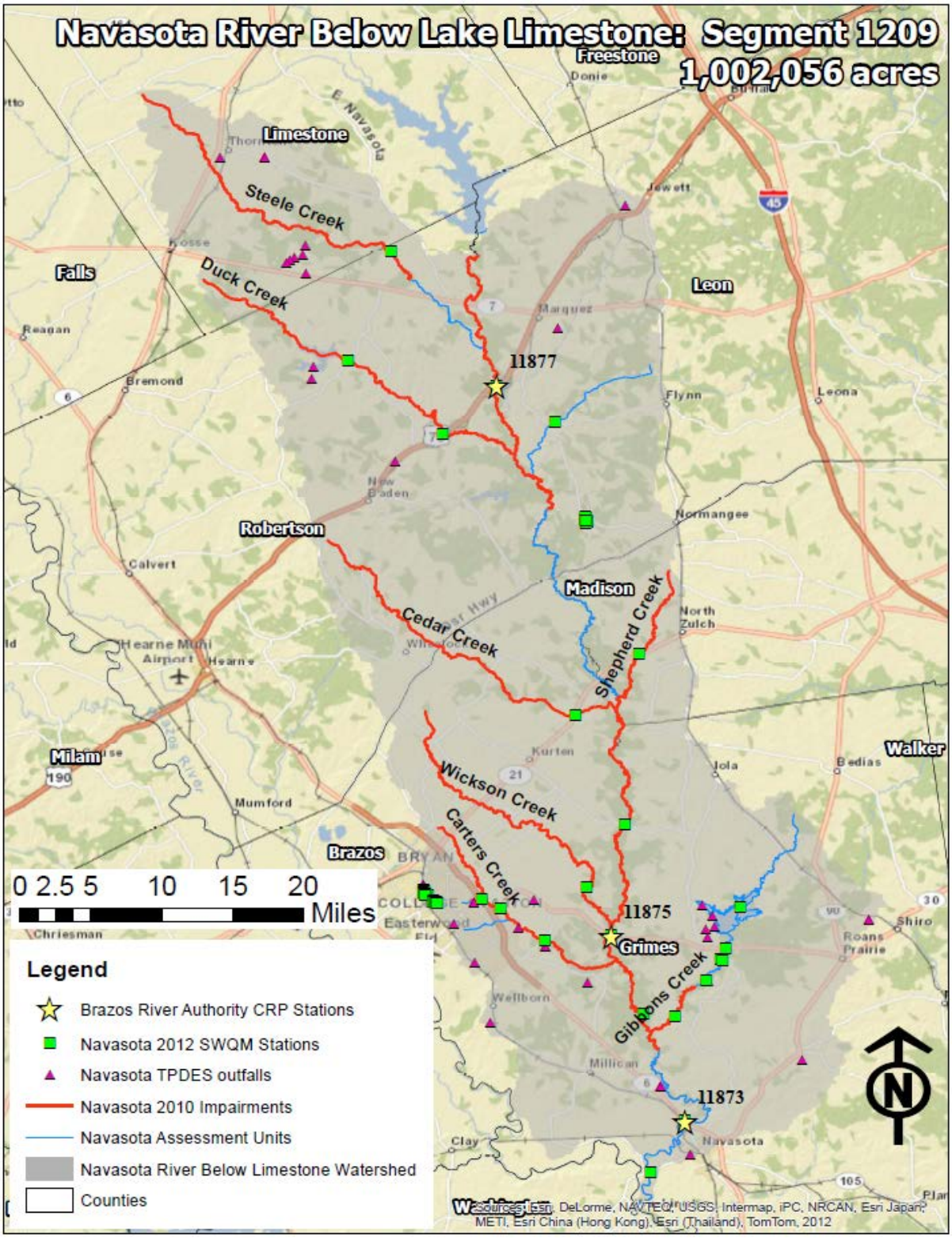


Figure A6-1. The Navasota River Watershed Below Lake Limestone

Table A6-1. Project Plan Milestones

Task	Project Milestones	Agency	Start Month	End Month
3.1	Gather existing *E. coli*, flow and other relevant water quality data for the basin from TCEQ, Clean Rivers Program (CRP), U.S. Geologic Survey (USGS), and other sources as appropriate	TWRI	2	18
3.2	Gather TPDES permit info for all permitted facilities	TWRI	2	8
3.3	Assess the number of existing water quality management plans in targeted basins and determine the current level and type of best management practice implementation existing	TWRI	2	8
3.4	Assemble existing GIS data and develop needed maps including: watersheds and subwatersheds, landuse/land cover, soils, topography, wastewater treatment facility locations, permitted confined animal feeding operation locations, geology, monitoring site locations, etc. as appropriate	TWRI	2	24
3.5	Assess OSSF numbers and locations using available information and will estimate OSSF densities in other areas of the watershed utilizing published methods	TWRI	2	18
3.6	Compile prior reports and publications if present and will glean relevant information from them related to pollutant loadings, sources and uses of the waterbody	TWRI	1	12
3.7	Develop LDCs for all sites in the watershed with adequate data to determine current loadings, total allowable load to meet standards, and the reductions needed to attain water quality standards	TWRI	6	18
3.8	Assess bacteria sources and potential pollutant contributions from those sources in the watershed using GIS-based methods that incorporate known or estimated animal populations and established methods for pollutant production	TWRI	6	18
4.1	Collect water samples and record stream flow when feasible from TCEQ Station 11875 located downstream of Highway 30 east of College Station bi-weekly for one year	TWRI	6	18
4.2	Enumerate *E. coli* in the 24 water samples collected using EPA method 1603.	SAML	6	18
4.3	Analyze 96 *E. coli* isolates from the 24 water samples using ERIC-PCR and RiboPrinting and compare results with known isolates from Texas *E. coli* BST Library to assess relative contributions from cattle, other livestock, wildlife, and humans. Each sample will also be processed to detect the presence or absence of known strains of *Bacteroidales* fecal bacteria.	SAML	6	18
4.4	Analyze 24 water samples received for nitrate, nitrite, ammonium, and orthophosphorus. These data will be non-regulatory and will not be uploaded into SWQMIS	NAWA	6	18
4.5	Develop and manage a waterbody specific database for storing collected water quality data. TWRI will also facilitate data transmittal to the TCEQ SWQMIS database and ensure that data are formatted consistent with the TCEQ DMRG.	TWRI	6	24

Load Duration Curves

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

(FDC) (Figure A6-2) uses the hydrograph of the observed stream flows to calculate and depict the percentage of time the flows are equaled or exceeded.

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

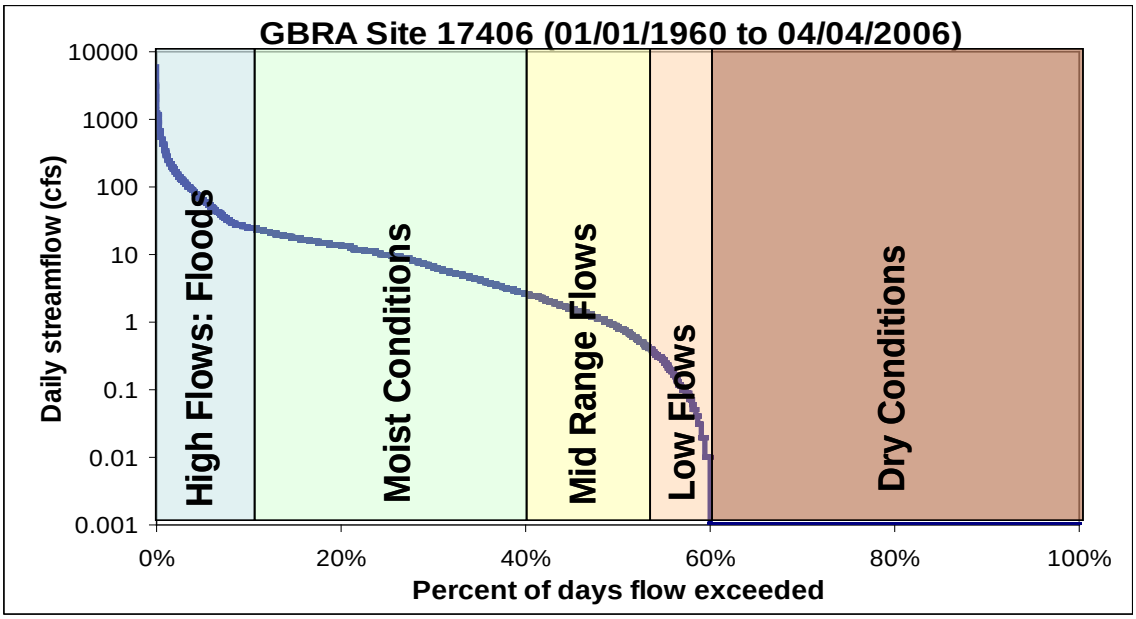


Figure A6-2. FDC for streamflow conditions at GBRA monitoring station 17406 on Plum Creek, near Uhland, TX. The flow data at 17406 was obtained from the nearest USGS gage station 8172400, after adjusting for subwatershed aerial contribution during runoff events.

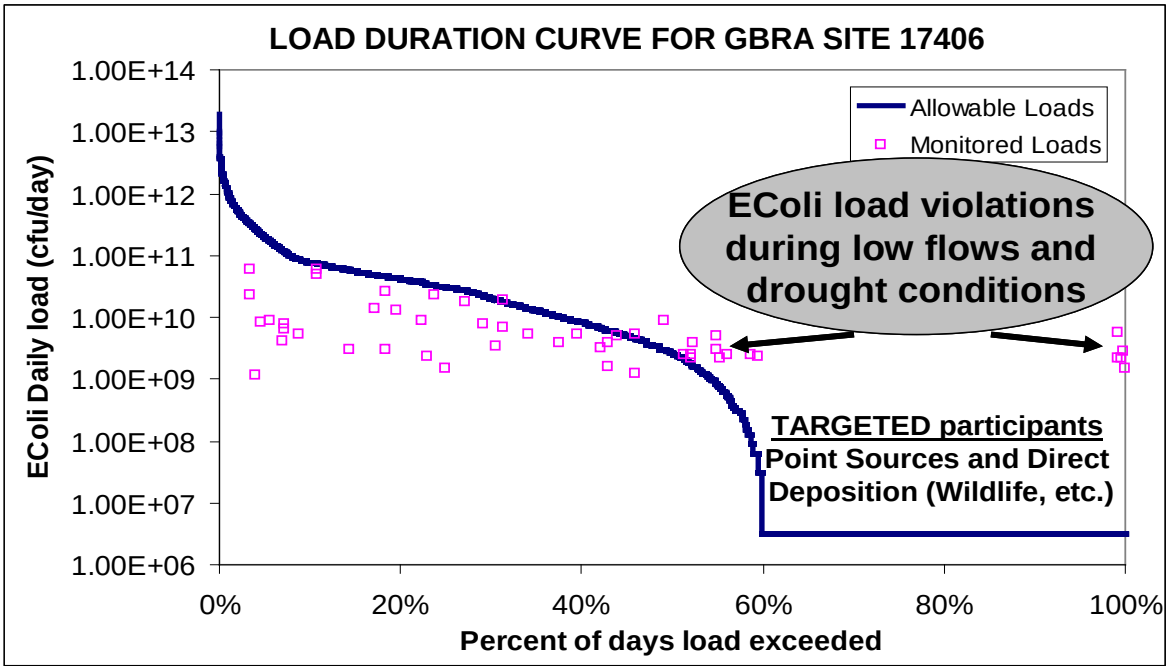


Figure A6-3. LDC for *E. coli* at GBRA monitoring station 17406 on Plum Creek, near Uhland, TX. The flow data at 17406 was obtained from the nearest USGS gage station 8172400, after adjusting for subwatershed aerial contribution during runoff events.

Surface Water Quality Monitoring

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

For *E. coli* enumeration and BST analysis purposes, the SAML will receive water samples and process them for *E. coli* isolation. The lab will also process water samples for *Bacteroidales* PCR analysis. *E. coli* will be isolated from the samples using standard microbiological methods as previously used in TSSWCB and TCEQ BST projects. *E. coli* will be isolated from water samples using USEPA Method 1603 and modified membrane Thermotolerant *E. coli* (mTEC) medium. The use of modified mTEC medium for isolation of *E. coli* from water will help avoid selection of different types of *E. coli* due to different media. Inoculated plates will be incubated at 35±0.5^{o}C for 2 hours to resuscitate stressed bacteria, then incubated at 44.5±0.2^{o}C for approximately 20 to 24 hours. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate. The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with 4-methylumbelliferyl-β-D-glucuronide (MUG) observed by ultraviolet light fluorescence.

Nutrient analysis will be carried out by the NAWA Lab to evaluate levels of nitrate and nitrite, ammonium, and orthophosphorus in water. NAWA will receive water samples and process them using USEPA methods 353.2, 350.1, and 365.1 respectively. These are colorimetric methods that use and automated instrument to record ambient nutrient levels.

Table A6-2. Sampling Site Description

Station ID	Station Name (Long Description)	GPS Coordinates	Monitoring Frequency
Routine Monitoring Stations			
11875	Navasota River Immediately Downstream of SH 30 East of College Station	30.607409; -96.181839	Bi-weekly

Bacterial Source Tracking

Limited Library Dependent BST

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

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

A total of 96 *E. coli* isolates obtained from ambient water samples from across the study area will be characterized using ERIC-PCR and RiboPrinting. DNA patterns of those isolates will be compared to the Texas *E. coli* BST Library of *E. coli* isolates from known animal and human sources collected throughout Texas. Water isolates will be identified to cattle, other livestock-avian, other livestock-non-avian, avian wildlife, non-avian wildlife, human (sewage), and pet sources (seven-way split), as well as a broader three-way split of livestock, human, and wildlife.

Library Independent BST

PCR genetic testing for *Bacteroides* fecal bacteria will be performed by SAML to determine the source of the fecal pollution. The *Bacteroidales* PCR method is a culture-independent molecular method which targets genetic markers of *Bacteroidales* and *Prevotella* spp. fecal bacteria that are specific to humans, ruminants (including cattle and deer), pigs, and horses (Bernhard& Field 2000; Dick et al. 2005). The method has high specificity and moderate sensitivity (Field et al. 2003). For this method, 100 ml water samples are concentrated by filtration, DNA extracted from the concentrate and purified, and aliquots of the purified DNA analyzed by PCR. For pre-processing of water samples for *Bacteroidales* PCR, SAML will filter the water samples, place the filters in DNA lysis buffer and freeze at -80° C until analysis. At the time of analysis, SAML will extract and purify DNA from the filters. DNA extracted from the water samples will be tested for the general, human, ruminant (including cattle and deer), pig (including feral hogs), and horse fecal markers. Results are typically expressed as presence/absence of the host-specific genetic markers; therefore, this method is not quantitative.

Section A7: Quality Objectives and Criteria for Model Inputs / Outputs

Faculty at TWRI and SCSC will conduct water quality monitoring a phased modeling effort to develop pollutant source and loading information and estimates of needed bacteria and nitrate reductions. The objectives of the water quality modeling for this project are as follows:

The objectives for this project are as follows:

1) Develop and obtain approval for a QAPP
2) Collect environmental and water quality data to support the development of a WPP
3) Utilize computer based programming to develop LDCs and evaluate contaminant loading potential using GIS
4) Utilize BST as a means to help direct bacteria targeted management measures that will be outline in the WPP

SWQM – The goal of this section is to ensure that data collected meets the DQOs of the project. The objective of this project is to identify the level and specific sources of bacteria and ammonia entering the Navasota River. Achievement of these objectives will support decisions for implementation of appropriate best management practices (BMPs) in order to reduce fecal bacteria levels in the Navasota River watershed to comply with existing water quality standards.

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

- Monitor water quality as related to bacterial and nutrient loading
- Determine the source of the bacterial impairment using BST

The measurement performance criteria to support the project objective are specified in Table A7-1.

Consistent with the most recent version of TCEQ's *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods* (TCEQ 2012b), routine grab samples will be collected on a bi-weekly (twice monthly) basis. During routine sampling measurements of DO, conductivity, pH, stream flow, and water temperature will be obtained *in situ*. These data will be logged on field data sheets, incorporated into a computer-based database maintained by TWRI.

Water samples collected will be transported to the NAWA Lab for nutrient analysis and SAML for bacteria enumeration and BST analysis. TWRI will deliver water samples to NAWA/SAML within designated holding times for respective analysis. Methods outlined in Tables A7-1 and B2-1 will be employed by each lab, as applicable. Appropriate DQOs and QA/QC requirements for this analysis are also reported in Tables A7-1 and B2-1.

LDC – this approach has been utilized in several TMDL and WPP projects as an initial screening-tool to evaluate the actual temporal load trends in streams (Cleland, 2003; Stiles, 2001). In cases of violations, it is necessary to determine the required load-reduction in that region near the monitoring station. Load-reductions should be calculated for all flow-regimes of

the stream. In order to do this continuous monitoring data will be simulated using the actual monitoring data by regression methods. Uncertainty of the model will be estimated via residual error analysis. The straight line passing through residual error plot should have a slope of zero.

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

Ambient Water Reporting Limits

Ambient water reporting limits (AWRLs) are reporting specifications at or below which data for a parameter must be reported to be compared with freshwater screening criteria. The AWRLs specified in Table A7-1 are the program-defined reporting specifications for each analyte and yield data acceptable to meet project objectives. The limit of quantitation (LOQ) is the minimum level, concentration, or quantity of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence. The laboratory is required to meet the following:

- The laboratory's LOQ for each analyte must be at or below the AWRL as a matter of routine practice.

- The laboratory will demonstrate and document its ability to quantitate at its LOQ for each analyte by running an LOQ Check Sample for each analytical batch of TMDL samples analyzed.

Acceptance criteria are defined in Section B5.

Table A7-1. Measurement Performance Specifications

Parameter	Units	Matrix	Method	Parameter Code	AWRL	Limit of Quantitation (LOQ)	Precision (RPD of LCS/LCSD)	Bias % Rec of LCS	LOQ Check Std % Rec	Lab
Field Parameters										
pH	Standard Units	water	EPA 150.1 and TCEQ SOP, V1[2]	00400	NA	NA	NA	NA	NA	Field
DO	mg/L	water	SM 4500-O G and TCEQ SOP, V1	00300	NA	NA	NA	NA	NA	Field
Specific Conductance, Field	µS/cm	water	EPA 120.1 and TCEQ SOP, V1	00094	NA	NA	NA	NA	NA	Field
Water Temperature	°C	water	SM 2550 B and TCEQ SOP, V1	00010	NA	NA	NA	NA	NA	Field
Transparency, Secchi disc	meters	water	TCEQ SOP, V1	00078	NA	NA	NA	NA	NA	Field
Turbidity	NTU	water	EPA 180.1	82078	NA	NA	NA	NA	NA	Field
Days since precipitation event	days	other	TCEQ SOP, V1	72053	NA	NA	NA	NA	NA	Field
Rainfall in 1 day inclusive prior to sample	inches	other	TCEQ SOP, V1	82553	NA	NA	NA	NA	NA	Field
Rainfall in 7 days inclusive prior to sample	inches	other	TCEQ SOP, V1	82554	NA	NA	NA	NA	NA	Field
Flow Stream, Instantaneous	cfs	water	TCEQ SOP, V1	00061	NA	NA	NA	NA	NA	Field
Flow measurement method	1-gage; 2-electric; 3-mechanical; 4-weir/ flume; 5-doppler	other	TCEQ SOP, V1	89835	NA	NA	NA	NA	NA	Field
Flow severity	1-no flow; 2-low; 3-normal; 4-flood; 5-high; 6-dry	water	TCEQ SOP, V1	01351	NA	NA	NA	NA	NA	Field
Present Weather	1-clear, 2-prt. Cloudy, 3-cloudy, 4-rain, 5-other	sky	TCEQ SOP, V1	89966	NA	NA	NA	NA	NA	Field
Depth to bottom of water at sample site	meters	other	TCEQ SOP, V1	82903	NA	NA	NA	NA	NA	Field
Water surface	1-calm; 2-ripples; 3-waves; 4-white caps	other	TCEQ SOP, V1	89968	NA	NA	NA	NA	NA	Field
Air Temperature	°C	air	TCEQ SOP, V1	00020	NA	NA	NA	NA	NA	Field
Primary Contact, Observed Activity (# of people)	NU	Other	TCEQ SOP, V1	89978	NA	NA	NA	NA	NA	Field
Evidence of Primary Contact Recreation	0-not observed; 1-observed	Other	TCEQ SOP, V1	89979	NA	NA	NA	NA	NA	Field
Bacteriological Parameters										
E. coli, mTEC	CFU/100 mL	water	EPA 1603	31648	1	1	0.5[1]	NA	NA	Lab

[1] Based on a range statistic as described in Standard Methods, 20th Edition, Section 9020-B, Quality Assurance/Quality Control -Intralaboratory Quality Control Guidelines. This criterion applies to bacteriological duplicates with concentrations >10 MPN/100mL or >10 organisms/100mL.

[2] Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue (RG-415) (revised August 2012).

Table A7-2. Measurement Performance Specifications for BST Analysis

Parameter	Method Type	Method	Method Description	Precision of Laboratory Duplicates	Bias	Percent Complete	Lab
E. coli _ RiboPrinting	DNA/image matching	Texas BST SOP	RiboPrinting	90% identical	90% correct	90	SAML
E. coli _ ERIC-PCR	DNA/image matching	Texas BST SOP	ERIC-PCR	90% identical	90% correct	90	SAML
Bacteriodales PCR	PCR Presence/Absence	Texas BST SOP	Bacteroidales PCR	100 % agreement	90% correct	90	SAML
E. coli Isolation	membrane filter culture on modified mTEC agar	EPA 1603	Membrane Filter	NA	NA	NA	SAML

Table A7-3. Measurement Performance Specifications for Nutrient Analysis

Parameter	Method Type	Method	Method Description	AWRL	Precision of Laboratory Duplicates	Bias	Percent Complete	Lab
Ammonium-N	Automated colorimetry	EPA 350.1	Colorimetric	.005 mg/L	<5% CV	±2%	90	NAWA
Nitrate-N	Automated colorimetry	EPA 353.2	Colorimetric	.005 mg/L	<5% CV	±2%	90	NAWA
Nitrite-N	Automated colorimetry	EPA 353.2	Colorimetric	.005 mg/L	<5% CV	±2%	90	NAWA
Orthophosphate-P	Automated colorimetry ascorbic acid	EPA 365.1	Colorimetric	.005 mg/L	<5% CV	±2%	90	NAWA

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

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

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

Representativeness
Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions. 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 and sampling of all pertinent media and use of only approved analytical methods will assure that the measurement data represents the conditions at the site.

Most data collected will be considered representative of ambient water quality conditions and will be coded with the applicable Monitoring Type Code specified in Table A9-2. The goal for meeting total representation of the water body is tempered by the availability of time and funding. Representativeness will be measured with the completion of samples collected in accordance with the approved QAPP and sampling plan.

The specific goal of this project is to collect samples that are representative of ambient water quality conditions and will not target any specific flow type. Sample collection will be carried out on a pre-planned, routine basis and will only be amended due to adverse weather conditions that effect sampling crew safety.

Comparability
Confidence in the comparability of data sets from this project and those for similar uses is based on the commitment of project staff to use only approved sampling and analysis methods and

QA/QC protocols in accordance with quality system requirements and as described in this QAPP and in TCEQ SOPs. Comparability is also guaranteed by reporting data in standard units, by using accepted rules for significant figures, and by reporting data in a standard format as specified in the most recent version of the SWQM DMRG.

Completeness

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

Section A8: Special Training Requirements/Certification

SWQM
Field personnel will receive training as described in the SWQM Procedures Manual (TCEQ 2012) in proper instrument calibration (Ch 8), sampling and field analysis (Chs 2, 3, 4, 5 where appropriate) and record keeping. Before actual sampling or field analysis occurs, they will demonstrate to the TWRI QA Officer their ability to properly calibrate field equipment and perform field sampling and analysis procedures. Training will be documented and retained in the TWRI personnel file and be available during an audit. Specific training received will include the proper operation, calibration and maintenance of YSI 556 MPS multiprobe, YSI EXO multiprobe, Son-Tek Flow Tracker, and the Son-Tek River Surveyor M9 as appropriate.

NAWA is responsible for analyzing water samples for nutrients. Data are for non-regulatory purposes and will not be included in SWQMIS. Laboratory personnel are all trained on appropriate instrumentation and have demonstrated their proficiency.

SAML will be responsible for analyzing bacteriological samples for inclusion in SWQMIS under this QAPP and is The NELAC Institute (TNI) certified for *E. coli* analysis using USEPA 1603 method and meets the requirements contained in TNI Volume 1 Module 2, Section 4.4 (2009) (concerning Review of Requests, Tenders, and Contracts).

LDC
TWRI Staff have previous experience in the development of FDCs/LDCs and have the needed knowledge/expertise to adequately perform this task.

GIS Inventory
TWRI Staff have past experience in the development of GIS inventories and has the needed knowledge/expertise to adequately perform this task.

BST
All personnel involved in sample analyses and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. SAML personnel involved in this project have been trained in the appropriate use of laboratory equipment, laboratory safety, cryogenics safety, and all applicable Texas BST SOPs. Laboratory analysts must demonstrate their capability to conduct each test that the analyst performs to the Lab Director. This demonstration of capability is performed before analyzing samples and annually thereafter. Finally, SAML is TNI certified for enumerating *E. coli* in both non-potable and drinking water using USEPA Method 1603.

Section A9: Documentation and Records

SWQM

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

LDC

All records, including modeler's notebooks and electronic files, will be archived by TWRI for at least five years. These records will document the source of historical data. Electronic data on the project computers and the network server are backed up daily to a tape drive. In the event of a catastrophic systems failure, the tapes can be used to restore the data in less than one day's time. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

BST

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

Combined Project Documentation

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

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

Table A9-1 lists documents, storage locations, retention times and forms.

Table A9-1. Project Documents and Records

Document/Record	Location	Retention	Form
QAPP, amendments, and appendices	TWRI	5 years	Electronic
QAPP distribution documentation	TWRI	5 years	Electronic
Corrective Action Reports (CARs)	TWRI	5 years	Electronic
Field notebooks & data sheets	TWRI	5 years	Paper/Electronic
Field equipment calibration & maintenance logs	TWRI	5 years	Paper/Electronic
Bacteria data log sheets	SAML	5 years	Paper/Electronic
Laboratory QA manuals	NAWA/SAML	5 years	Paper/Electronic
Laboratory methods guidance	NAWA/SAML	5 years	Paper/Electronic
Instrument raw data files, readings, printouts	NAWA/SAML	5 years	Paper/Electronic
Laboratory equipment calibration records & maintenance logs	NAWA/SAML	5 years	Paper/Electronic
Lab data reports	NAWA/SAML/TWRI	3 years	Paper/Electronic
Progress reports, final reports, data	TWRI/TSSWCB	3 years	Paper/Electronic

Laboratory records must be retained in accordance with TNI standards (2009). The TSSWCB may elect to take possession of records at the conclusion of the specified retention period.

QAPP Revision

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

Amendments

Amendments to the QAPP may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and non-conformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances. Requests or amendments are directed from the TWRI Project Lead to the TSSWCB PM in writing. The changes are effective immediately upon approval by the TSSWCB PM and Quality Assurance Officer, or their designees. Amendments to the QAPP and the reasons for the changes will be documented, and copies of the approved QAPP Expedited Amendment form will be distributed to all individuals on the QAPP distribution list by the TWRI QAO. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

Field Data Sheets
When data are collected in the field, field data sheets will be used. A composite field data sheet has been developed to support all three types of monitoring. A copy of this field data sheet is included in Appendix B. Following each sampling event, field data sheets will be turned into the TWRI PM or their representative for storage.

Laboratory Data Reports
Test/data reports from the laboratory must document the test results clearly and accurately. Routine data reports should be consistent with *TNI Volume 1 Module 2 Section 5.10* (2009) and include the information necessary for the interpretation and validation of data. The requirements for reporting data and the procedures are provided. Information documented will include the following at a minimum:

- Sampling Location
- Station ID
- Date/Time Collected
- Name of Sample Collector
- Date/Time Received
- Name of Sample Receiver
- Analysis Performed
- Units of measurement
- Sample Volume Processed
- Name of Person Processing Sample and Recording Results
- Sample Analysis Results
- Narrative of any QA/QC deviations or failures that may affect sample quality
- Certification of TNI compliance

Electronic Data
TWRI will use the electronic data reporting formats included in the most recent version of the SWQM DMRG. A completed Data Review Checklist (see Appendix D) will accompany each set of electronic data.

Only data that meet the measurement performance specifications in Table A7-1 and Section B5 will be included in electronic data files destined for uploading into the TCEQ's SWQMIS database. Also, data flagged with qualifiers found in Appendix E of the SWQM DMRG will not be submitted in the electronic files that will be uploaded into the SWQMIS database.

Data submitted to the TCEQ that are not also submitted for entry into SWQMIS will be identified in a separate text file that is submitted to the TSSWCB PM. This file will include written explanations as to why the data were not submitted for entry into the SWQMIS database.

All reported Events will have a unique TagID (see DMRG). TagIDs used in this project will be seven-character alphanumeric with a structure consisting of a two-letter Tag prefix (TX) followed by a five digit number: for example – TX00001, TX00002, etc.

Submitting Entity, Collecting Entity, and Monitoring Type codes will reflect the project organization and monitoring type in accordance with the DMRG as shown in Table A9-2. The proper coding of Monitoring Type is essential to accurately capture any bias toward certain environmental condition (for example, high flow events). The TSSWCB PM will be consulted to assure proper use of the Monitoring Type code.

Table A9-2. SWQMIS Data Entry Codes

Sample Description	Tag Prefix	Submitting Entity	Collecting Entity	Monitoring Type
Routine monitoring to establish baseline conditions	*TX*	*TX*	*WR*	*RTWD*

Section B1: Sampling Process Design (Experimental Design)

SWQM

Data collection and analysis will play a pivotal role in this project and will provide data to inform SWCDs and landowners of any potential or existing water quality issues and/or problems and form the foundation for developing the Navasota River WPP. In addition, water samples will be analyzed to determine the source of bacteria entering the stream. This information will be instrumental in evaluating potential BMPs to implement in the watershed as well as aid in WPP development. Achievement of these objectives will support decisions on how to best target management measures to reduce fecal bacteria levels in the Navasota River watershed. Parameters to be measured are shown in Table A7-1. All data to be collected under this project are considered critical.

The sampling program is designed to characterize water quality of all flow conditions in the Navasota River. Water quality grab samples will be routinely collected on bi-weekly (twice monthly) intervals for all constituents as directed by TCEQ (2012b). All samples will be collected routinely (e.g. as scheduled and not targeted to any specific flow condition) for a one year period at a single sampling station on the Navasota River at State Highway 30. In total, 24 samples are expected from this monitoring effort. Physical parameters that will be measured *in situ* during routine sampling include flow (cfs), specific conductance, DO, pH, and water temperature; other noted items will include the flow severity, days since last significant rainfall, occurrence of contact recreation, evidence of contact recreation and present weather conditions. Water quality samples collected as part of the routine sampling schedule will be analyzed for bacteria and nutrients as outlined in Table A7-1.

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

In the instance that the sampling site (Table A6-2) is inaccessible, no sample will be taken and will be documented in the field notebook. If, near the end of the study, the TSSWCB PM/QAO agrees that the sampling has not achieved good representativeness of typical conditions, the final sampling event(s) may be extended.

BST

To provide sufficient water quality data to characterize bacteria loadings, routine ambient monitoring will be conducted by TWRI at a single stream site (see Table A6-2). Field data and samples will be collected following procedures detailed in the most recent version of TCEQ (2012b).

Samples collected by TWRI will be delivered to the lab at NAWA and SAML for processing and analysis. SAML will perform Bacteroidales PCR on approximately 24 individual water

samples collected by TWRI. SAML will also isolate and fingerprint ERIC-RP *E. coli* (four per site per sample event) resulting in a total of 96 individual samples analyzed using ERIC-PCR.

Section B2: Data Collection Methods

SWQM

TWRI will follow the most recent versions of the field sampling procedures documented in the TCEQ (2012b).

Water samples will be collected directly from the stream (midway in the stream channel) in most cases. Water samples used for *E. coli* analysis will be collected in sterile 200 mL Whirl-Pak bags. All sample containers will be labeled with the following information:

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

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

Water temperature, pH, specific conductivity, specific conductance, and dissolved oxygen will be measured and recorded *in situ* with a multiprobe whenever samples are collected. Flow is measured with an acoustic Doppler velocimeter as described in the TCEQ (2012b). All field measurements will be conducted in accordance with the methods listed in Table B7-1. All samples will be transported in a container with ice to the laboratory for analysis.

Sample Volume, Container Types, Minimum Sample Volume, Preservation Requirements and Holding Time Requirements

Table B2-1. Field Sampling and Handling Procedures

Parameter	Matrix	Container	Preservation (includes Ice)	Sample Volume	Holding Time
E. coli, MTEC	Water	Sterile Whirl-Pak Bag	Ice, ≤6°C	~150 mL	6 hours
Nitrate-N	Water	Sterile Whirl-Pak Bag	Ice, ≤6°C	~150 mL	20 hours
Nitrite-N	Water	Sterile Whirl-Pak Bag	Ice, ≤6°C	~150 mL	20 hours
Ammonium-N	Water	Sterile Whirl-Pak Bag	Ice, ≤6°C	~150 mL	24 hours
Ortho-Phosphate-P	Water	Sterile Whirl-Pak Bag	Ice, ≤6°C	~150 mL	24 hours

Processes to Prevent Contamination

The most recent version of the TCEQ (2012b) outlines the necessary steps to prevent contamination of samples. These include: direct collection into sample containers, when possible. Field QC samples as discussed in Section B5 are collected to verify that contamination has not occurred.

Documentation of Field Sampling Activities

Field sampling activities are documented on field data sheets as presented in Appendix B. Flow work sheets, multi-probe calibration records, and records of bacteria analyses *(if applicable)* are part of the field data record. For all visits, station ID, location, sampling time, sampling date, sampling depth, and sample collector's name/signature are recorded. Values for all measured field parameters are also recorded. Detailed observational data are recorded as well, including: water appearance, weather, biological activity, stream uses, watershed or in stream activities, unusual odors, specific sample information, missing parameters, days since last significant rainfall, 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:

1. Write legibly in indelible ink;
2. Changes should be made by crossing out original entries with a single line, entering the changes, and initialing and dating the corrections;
3. Close-out incomplete pages with an initialed and dated diagonal line.

Sampling Method Requirements or Sampling Process Design Deficiencies, and Corrective Action

Examples of sampling method requirements or sample design deficiencies include but are not limited to such things as inadequate sample volume due to spillage or container leaks, failure to preserve samples appropriately, contamination of a sample container during collection, storage temperature and holding time exceedance, sampling at the wrong site, etc. Any deviations from the QAPP and appropriate sampling procedures may invalidate resulting data and will require corrective action to prevent future recurrences. Corrective action may include for samples to be discarded and re-collected. It is the responsibility of the TWRI PM, in consultation with the TWRI QAO, to ensure that the actions and resolutions to the problems are documented and that records are maintained in accordance with this QAPP. In addition, these actions and resolutions will be conveyed to the TSSWCB PM in writing in the project progress reports and by completion of a CAR.

The definition of and process for handling deficiencies, nonconformance and corrective actions are defined in Section C1.

BST

See SWQM section on previous pages.

Section B3: Sample Handling and Custody Requirements

SWQM & BST

Sample Tracking

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

A sample is in custody if it is in actual physical possession or in a secured area that is restricted to authorized personnel. The COC form is a record that documents the possession of the samples from the time of collection to receipt in the laboratory. The list of items below is included on the COC form (See Appendix C for sample form).

1. Date and time of sample collection, shipping and receiving
2. Site identification
3. Sample matrix
4. Number of containers
5. Preservative used
6. Analyses required
7. Name of collector
8. Custody transfer signatures and dates and time of transfer

Sample Labeling

Samples are labeled on the container with an indelible, waterproof marker. Label information includes:

1. Site identification
2. Date and time of collection
3. Sampler initials
4. Sample type (i.e., analyses) to be performed

Sample Handling

Upon collection, sealing of the sample and following proper labeling, water samples are placed in an insulated cooler on ice and transported to the designated lab along with appropriate COCs within prescribed holding times. Routine samples will be delivered to SAML while reconnaissance samples will be returned to TWRI for processing. Once at the lab, samples and COCs are transferred to lab staff, are logged into the lab and analysis/bench sheets (see Appendix F) specific to the respective laboratory are established for each sample. Samples are placed in a refrigerated cooler dedicated to sample storage until sample processing begins. The Laboratory Director has the responsibility to ensure that holding times are met with water samples. The holding time is documented on the COC.

Sample Tracking Procedure Deficiencies and Corrective Action

All failures associated with chain-of-custody procedures as described in this QAPP are immediately reported to the TWRI 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 TWRI PM in consultation with the TWRI QAO will determine if the procedural violation may have compromised the validity of the resulting data. Any failures that have reasonable 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 PM in the project progress report. CARs will be prepared by the TWRI QAO and submitted to the TSSWCB PM along with project progress reports.

Section B4: Analytical Methods

SWQM

The analytical methods associated matrices, and performing laboratories are listed in Table A7-1 of Section A7. Procedures for laboratory analysis must be in accordance with the most recently published edition of the book entitled *Standard Methods for the Examination of Water and Wastewater*, the TCEQ Surface Water Quality Monitoring Procedures, 40 CFR 136, the TST Water Quality Monitoring Manual or other reliable procedures acceptable to the commission. Exceptions to this include analyses and sample matrices for which no regulated methods exist, or where EPA has not approved any method with adequate sensitivity for outlined data requirements. Data that are collected or analyzed by means other than those stated in the QAPP, or data that are suspect for any reason, should not be submitted for loading and storage in SWQMIS. In this project, these methods include the USEPA 1603 modified mTEC method for *E. coli* enumeration.

SAML will analyze samples under this QAPP and is accredited by TCEQ and TNI to perform analyses using the methods listed in Table A7-1 in non-potable water. Copies of laboratory QAMs and SOPs are retained by the laboratory and are available for review by the TCEQ. Laboratory SOPs are consistent with EPA requirements as specified in the method.

Nutrient samples will be processed for informational purposes only and will not be submitted to TCEQ's SWQMIS database. These samples will be processed by the NAWA lab, as noted below.

Nitrate-Nitrite-N in water samples will be analyzed in the NAWA lab using USEPA Method 353.2 [Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry, Method 353.2; USEPA 353.2-1. 1993]. This method determines nitrate-N singly or nitrate-N and nitrite-N combined. Nitrate-N is determined colorimetrically by subtracting the nitrate reading from the nitrite-N reading, which is produced from nitrate reduced to nitrite by passing through a copper-cadmium granule column.

Ammonium-N in water samples will be analyzed in the NAWA lab using USEPA Method 350.1 [Determination of Ammonium Nitrogen by Semi-Automated Colorimetry, Method 350.1; USEPA 350.1-1. 1993]. This method determines ammonium-N by buffering and distilling the sample into boric acid, which forms a blue color that is measured colorimetrically.

Ortho-phosphate-P in water samples will be analyzed in the NAWA lab using USEPA Method 365.1 [Phosphorous, All Forms. Method 365.1 (Colorimetric, Automated, Ascorbic Acid). Pp.365-1.1 – 365-1.7. In *Methods for Chemical Analysis of Water and Wastes.* USEPA-600/ 4-79-020. 1983.]. Ortho-phosphate-P concentrations are determined through the ascorbic acid/molybdate blue method.

BST

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

E. coli in water samples will be quantified and isolated by SAML using modified mTEC agar, EPA Method 1603 (USEPA 2009). The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with MUG observed by ultraviolet light fluorescence.

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

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

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

Standards Traceability

All standards used in the field and laboratories are traceable to certified reference materials. The majority of standards utilized are purchased as fully prepared, traceable standards. Where appropriate, standards preparation is fully documented as describe in lab SOPs and maintained in a standards log book. Each documentation includes information concerning the standard identification, starting materials, including concentration, amount used and lot number, date prepared, expiration date and preparer's initials or signature. The reagent bottle will be labeled in a way that will trace the reagent back to preparation.

Analytical Method Deficiencies and Corrective Actions

Failures in field and laboratory measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, quality control samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst

will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst without compromising data quality, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable without compromising data quality, then it is conveyed to the SAML Supervisor, who will make the determination and notify the TWRI QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TCEQ. The nature and disposition of the problem is reported on the data report which is sent to the TWRI PM. The TWRI PM will include this information in the CAR and submit with the Progress Report which is sent to the TSSWCB PM.

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

Table B4-1. Analytical Methods

Laboratory Parameter	**Method**	**Equipment Used**
E. coli ERIC-PCR fingerprint	SAML SOP	PCR thermal cycler, gel electrophoresis app
E. coli RiboPrint fingerprint	SAML SOP	RiboPrinter
E. coli in water	USEPA 1603	Filtration apparatus, incubator
Ortho-phosphate=P	USEPA 365.1	Unity Scientific, SmartChem 200
Ammonium-N	USEPA 350.1	Unity Scientific, SmartChem 200
Nitrate-N	USEPA 353.2	Unity Scientific, SmartChem 200
Nitrite-N	USEPA 353.2	Unity Scientific, SmartChem 200
Field Parameter	**Method**	**Equipment Used**
pH	USEPA 150.1	YSI 556 MPS or EXO1 Sonde
DO	SM4500-O G	YSI 556 MPS or EXO1 Sonde
Specific Conductivity	USEPA 120.1	YSI 556 MPS or EXO1 Sonde
Temperature	SM2550B	YSI 556 MPS or EXO1 Sonde
Flow Rate (cfs)	TCEQ SOP V1	SonTek Flow Tracker or M9 River Surveyor

Failures in Measurement Systems and Corrective Actions

Failures in measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, QC samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the SAML Director, who will make the determination in coordination with the TWRI QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB as part of this project. The nature and disposition of the problem is reported on the data report. The TWRI QAO will include this information in the CAR and submit with the QPR which is sent to the TSSWCB PM.

Section B5: Quality Control Requirements

SWQM

The minimum Field QC Requirements are outlined in the TCEQ (2012b). Specific requirements are outlined below. Field QC samples are reported with the laboratory data report (See Section A9 and C2).

Table B5-1. Required Quality Control Analyses

Parameter	Matrix	LOQ	LOQ Check Standard	LCS	Lab Dup	Field Blank	Method Blank
***E. coli* Source Assessment Samples**							
E. coli ERIC-PCR	*Water*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
E. coli RiboPrint	*Water*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
E. coli	*Water*	*NA*	*NA*	*NA*	√	*NA*	√
Nutrient Samples							
Ortho- Phosphate-P	*Water*	√	√	√	√	√	√
Ammonium-Nitrogen	*Water*	√	√	√	√	√	√
Nitrate-Nitrogen	*Water*	√	√	√	√	√	√
Nitrite-Nitrogen	*Water*	√	√	√	√	√	√

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

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

Method Specific QC requirements – QC samples other than those specified later in this section are run as specified in the methods. The requirements for these samples, their acceptance criteria or instructions for establishing criteria, and corrective actions are method-specific. Sample duplicates, surrogates, internal standards, continuing calibration samples, interference check samples, positive control, negative control, and media blanks are examples of method-specified QC samples which are not discussed in this section.

Detailed laboratory QC requirements are contained within each individual method and laboratory quality assurance manuals (QAMs). The minimum requirements that all participants abide by are stated below. Lab QC sample results are reported with the laboratory data report (see Section C2 and A9).

LOQ Check Standard

An LOQ check standard consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system at the lower limits of analysis. The LOQ check standard is spiked into the sample matrix at a level less than or near the LOQ for each analyte for each analytical batch of samples run.

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

$$\%R = SR/SA * 100$$

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

Laboratory Control Sample (LCS)

An LCS consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system. The LCS is spiked into the sample matrix at a level less than or near the mid-point of the calibration for each analyte. In cases of test methods with very long lists of analytes, LCSs are prepared with all the target analytes and not just a representative number, except in cases of organic analytes with multi-peak responses.

The LCS is carried through the complete preparation and analytical process. LCSs are run at a rate of one per preparation batch. Results of LCSs are calculated by percent recovery (%R), which is defined as 100 times the measured concentration, divided by the true concentration of the spiked sample. The following formula is used to calculate percent recovery, where %R is percent recovery; SR is the measured result; and SA is the true result:

$$\%R = SR/SA * 100$$

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

Laboratory Duplicates

A laboratory duplicate is prepared by taking aliquots of a sample from the same container under laboratory conditions and processed and analyzed independently. A laboratory control sample duplicate (LCSD) is prepared in the laboratory by splitting aliquots of an LCS. Both

samples are carried through the entire preparation and analytical process. LCSDs are used to assess precision and are performed at a rate of one per preparation batch.

For most parameters, precision is calculated by the relative percent difference (RPD) of LCS duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X_1 and X_2, the RPD is calculated from the following equation:

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

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the lab. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair. Measurement performance specifications are used to determine the acceptability of duplicate analyses as specified in Table A7-1. The specifications for bacteriological duplicates in Table A7-1 apply to samples with concentrations > 10 organisms/100mL.

Method Blank
A method blank is a sample of matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. The method blank is carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the LOQ. For very high-level analyses, the blank value should be less than 5% of the lowest value of the batch, or corrective action will be implemented.

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

BST
Table A7-1 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the SCSC Project Leader to verify that the data are representative. The SCSC Project Leader also has the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of CARs (Appendix A). Laboratory audits, sampling site audits, and QA of field sampling methods will be conducted by the TSSWCB QAO or their designee.

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

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

Laboratory Duplicate
Laboratory duplicates are used to assess precision. A laboratory duplicate is prepared by splitting aliquots of a single sample (or a matrix spike or a laboratory control standard) in the laboratory. Both samples are carried through the entire preparation and analytical process. Laboratory duplicates are run at a rate of one per batch. Acceptability criteria are outlined in Table A7-1 of Section A7.

Precision is calculated by the RPD of duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X_1 and X_2, the RPD is calculated from the following equation:

$$\mathbf{RPD} = \frac{(X_1 - X_2) \times 100}{(X_1 + X_2) \div 2}$$

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the laboratory. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair.

Performance limits and control charts are used to determine the acceptability of duplicate analyses. Precision limits for bacteriological analyses are defined in Table A7-1 and applies to samples with concentrations >10 cfu/100 ml.

Failures in Quality Control and Corrective Action
Sampling QC excursions are evaluated by the TWRI PM, in consultation with the TWRI QAO. In that differences in sample results are used to assess the entire sampling process, including environmental variability, the arbitrary rejection of results based on pre-determined limits is not practical. Therefore, the professional judgment of the TWRI PM and QAO will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility.

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

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

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

All sampling equipment testing and maintenance requirements are detailed in the TCEQ (2012b). Sampling equipment is inspected and tested upon receipt and is assured appropriate for use. Equipment records are kept on all field equipment and a supply of critical spare parts is maintained.

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

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

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

Section B7: Instrument Calibration and Frequency

SWQM

Field equipment calibration requirements are contained in Chapter 8 of the TCEQ (2012b). Post calibration error limits and the disposition resulting from error are adhered to. Data not meeting post-error limit requirements invalidates associated data collected subsequent to the pre-calibration and are not submitted to the TCEQ.

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

BST

All instruments or devices used in obtaining environmental data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration.

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

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

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

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

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)

SWQM

Any non-direct measurements will comply with all requirements under this QAPP. Sampling conducted by the TCEQ, the USGS and Texas Clean Rivers Program partners is not covered under this QAPP and will not be reported to the TSSWCB PM by the TWRI. However, data collected by the above organizations that meet the data quality objectives of this project will be useful in satisfying the data and informational needs of the project. The collection and qualification of the TCEQ and USGS data are addressed in the TCEQ Surface Water Quality Monitoring QAPP. The collection and qualification of the Texas CRP data are addressed in the Texas Clean Rivers Program QAPPs. Historic water quality data collected through TCEQ's CRP program and under its approved QAPP will be utilized in this project. Parameters utilized will include instantaneous stream flow, temperature, pH, specific conductivity, DO, nitrate, ammonium, orthophosphorus, total phosphorus, and *E. coli* as available. Potential sites where data will be acquired from are included in Table B9-1. No limitations will be placed on these data as they have been vetted by the TCEQ SWQM Data Management and Assessment Team and were collected under a TCEQ approved QAPP.

Only data collected directly under this QAPP will be submitted to the TCEQ for storage in SWQMIS. This project will not submit any acquired or non-direct measurement data to SWQMIS that has been or is going to be collected under another QAPP. All data collected under this QAPP and any acquired or non-direct measurements will comply with all requirements/guidance of the project.

LDC

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

GIS Inventory

Geospatial data available from various local, regional, state, and federal organizations may be used for cartographic purposes. Maps developed for reports will be for illustrative purposes. Geospatial data utilized in maps of the study area may include land use, precipitation, soil type, ecoregion, TCEQ monitoring location, TCEQ permitted outfall, gage location, city/county/state boundary, stream hydrology, reservoir, drought, road, watershed, municipal separate storm sewer system, urbanized area, basin, railroad, recreational area, area landmark, aerial photography, and park information. The above data come from the following reliable sources: USGS, TNRIS, TCEQ, TXDOT, TSSWCB, TWDB, and US Census Bureau. Geospatial data from these sources

are accepted for use in this project maps based on the reputability of these data sources and the fact that there are no known comparable sources for these data. Geospatial data will be cited in reports.

Other data that are compiled and published by other entities may also be used in preparing project reports. This may include long-term precipitation, census, ecoregion, land use and land cover, historic water quality and stream flow data. Sources of these data are the USGS, National Weather Service, US Census Bureau, USDA NRCS, TCEQ, and TPWD. Data collected by these entities are assumed to have been verified and validated according to the requirements of the respective programs. Data compilations created for this project will be visually screened for errors. Data will be cited in reports.

Table B9-1 lists the type of measurement, data, units, source, QA documentation use and data range of each acquired data set where applicable.

BST Analysis
Data analyzed using BST analysis methods for this project will consist of data produced during the course of this study under the specifics of this QAPP, or generated under previous TSSWCB studies with accepted QAPPs.

Table B9-1. Non-Direct Data Types and Data Sources for the Waterbodies in the Navasota River Basin

Type of Measurement or Analysis	Type of Data (time series, rate, constant, statistic, taxa, etc.)	Units	Source (weblink when available)	Quality Assurance Documentation	Use	Date Range
Streamflow	Time series, daily streamflow	Average daily (cfs)	USGS http://waterdata.usgs.gov/tx/nwis/sw	Data noted as "Approved" (quality-assured data) or "Provisional" (of unverified accuracy and subject to revision). More recent “provisional” data may be used in the project after thorough review. ”Approved” data have successfully undergone USGS quality assurance.	FDCs	All data available
E. coli, specific conductance, nitrate, phosphorous, DO, instantaneous flow	Concentration at various points in time	CFU or MPN/100mLfor bacteria; µmhos/cm for spec. cond; ppm for nutrients; mg/L for DO, cfs for flow	TCEQ SWQMIS http://www.tceq.texas.gov/waterquality/data-management/wdma_forms.html	Data requested will include only data that met quality assurance/quality control (QA/QC) requirements as outlined under the SWQM Data Management Reference Guide.	LDCs	most recent 7 years; or 10 years if insufficient data exists
TCEQ Surface Water Quality Monitoring Stations	Spatial data, location of active and historical SWQM stations	Shapefile - Points	TCEQ GIS Site Layers Download Page http://www.tceq.texas.gov/gis/sites.html	Data Management Reference Guide (DMRG) for Surface Water Quality Monitoring http://www.tceq.texas.gov/waterquality/data-management/dmrg_index.html	Map development and FDCs/LDCs	N/A
TCEQ Segments	Spatial data, official TCEQ Segments	Shapefile - Polylines	TCEQ GIS Hydrology Layers http://www.tceq.texas.gov/gis/hydro.html	TCEQ 2010 Stream Segments Metadata http://www.tceq.texas.gov/assets/public/gis/metadata/stream_segments.pdf	Map development	N/A
County Boundaries	Spatial data, StratMap Boundaries	Shapefile - Polygons	TNRIS Data Search & Download http://www.tnris.org/	Metadata available with download	Map development	N/A
Watershed topography	Spatial GIS data, Digital Elevation Models (DEMs)	Raster- 10 meter resolution	National Elevation Dataset from USGS National Map Viewer http://nationalmap.gov/viewer.html	Digital Elevation Model Technologies and Applications: The DEM Users Manual 2nd Edition	Delineation of watershed and subwatershed boundaries for maps	N/A

Type of Measurement or Analysis	Type of Data (time series, rate, constant, statistic, taxa, etc.)	Units	Source (weblink when available)	Quality Assurance Documentation	Use	Date Range
Land Use/Land Cover	National Land Cover Dataset – GIS raster dataset	Raster – 30 m resolution	National Land Cover Database 2011 (NLCD2011) from MRLC Consortium Viewer http://www.mrlc.gov/nlcd2011.php	Jin, S., Yang, L., Danielson, P., Homer, C., Fry, J., and Xian, G. 2013. A comprehensive change detection method for updating the National Land Cover Database to circa 2011. *Remote Sensing of Environment*, 132: 159 – 175.	Map development	Based on Landsat imagery between 2001 and 2006
Soil Map Unit Boundaries and Properties	Spatial GIS data, Soils	Shapefile - polygons	NRCS SSURGO databases via Web Soil Survey http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm or Geospatial Data Gateway http://datagateway.nrcs.usda.gov/	SSURGO/STATSGO2 Structural Metadata and Documentation http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/ref/?cid=nrcs142p2_053631	Map development	various
Sanitary Sewer Overflows (SSOs)	Individual events	Location and amount (gallons)	TCEQ Regions 12 & 14 Excel database provided upon request by regional staff	Data entry based on reported occurrences, Level of QA unknown	Quantify reported SSOs	2000-2013
Municipal & Industrial WWTF Discharge Monitoring Reports	Self-reporting monthly discharge and concentration data	concentration bacteria (MPN/100mL or colonies/100mL), flow (MGD)	USEPA Enforcement & Compliance History Online (ECHO) website http://echo.epa.gov/echo/ or directly from permitted facilities	Reporting data based on permit requirements	Source analysis; FDCs/LDCs	2000 - present for presently active permits
General permits involving regulation of stormwater	Regulated entities	N/A	TCEQ Information Resources Division Central Registry http://www2.tceq.texas.gov/wq_dpa/index.cfm	None accessible; TCEQ databases	Determination of regulated stormwater for TMDL development	2000 - present
Water Rights Diversion Points	Spatial GIS and Tabular Data	N/A	TCEQ http://www.tceq.state.tx.us/gis/sites.html	None accessible; TCEQ databases	Understanding uses of surface water in the watershed	2013
Urbanized Areas	Spatial GIS	Shapefile - polygons	U.S. Census Bureau TIGER/Line® Shapefiles http://www.census.gov/cgi-bin/geo/shapefiles2010/main and information from municipalities	Urban-Rural Classification Program http://www.census.gov/geo/reference/urban-rural.html	Map development; define regulated stormwater	2010

Type of Measurement or Analysis	Type of Data (time series, rate, constant, statistic, taxa, etc.)	Units	Source (weblink when available)	Quality Assurance Documentation	Use	Date Range
Population	Spatial GIS and tabular data	2010 Census blocks, Shapefile – polygons	US Census Bureau, 2010 TIGER/Line® Shapefiles download interface http://www.census.gov/cgi-bin/geo/shapefiles2010/main; Tabular data from US Census Bureau, American Fact Finder http://factfinder2.census.gov/faces/nav/jsf/pages/index.xhtml	Metadata available with download	Map and source development	2010
Building locations	Spatial GIS, point data	Shapefile - points	Brazos Valley and Heart of Texas Councils of Government 911 address shapefiles	Programmatic	Map and source development, OSSF estimations	N/A
Hydrography	Vector GIS data	Geodatabase – points, polylines, polygons	National Hydrography Dataset (NHD)Pre-staged Subregions http://nhd.usgs.gov/data.html	NHD Program Documentation http://nhd.usgs.gov/program_documentation.html	Map development	N/A
Livestock population estimates	County-level livestock density	County level individual animals	USDA Census of Agriculture http://www.agcensus.usda.gov/	Regulations Guiding NASS http://www.agcensus.usda.gov/About_the_Census/Regulations_Guiding_NASS/index.php	Map and source development	2002-2012 (when available)
Deer	Spatial wildlife density	Density (animal per unit area)	Texas Parks & Wildlife Department surveys and/or information from biologists	Jester & Dillard (undated)	Source development	N/A
Cats and dogs	Spatial, pet density	number per household	AVMA 2002 U.S. Pet Ownership data and stakeholder input	[AVMA] American Veterinary Medical Association. 2002. U.S. Pet Ownership and Demographics Source Book.Schaumberg (Illinois): Center for Information Management, American Veterinary Medical Association.	Source development	N/A
Feral hogs	Spatial feral animal density	Feral hog density (animals per unit area)	TWRI, http://twri.tamu.edu/reports/2009/tr347.pdf TPWD, literature values and stakeholder input	Mellish et al. 2013.	Source development	N/A
Water and sewer service areas	Spatial GIS data	Shapefile - polygons	TCEQ GIS Regulatory/ Administrative Boundaries, Water & Sewer Certificates of Convenience and Necessity Service Areas,http://www.tceq.texas.gov/gis/boundary.html	Sewer CCN Service Areas Metadata, http://www.tceq.texas.gov/assets/public/gis/metadata/ccn_sewer.pdf	Map and source development	Present

Type of Measurement or Analysis	Type of Data (time series, rate, constant, statistic, taxa, etc.)	Units	Source (weblink when available)	Quality Assurance Documentation	Use	Date Range
Population projections	Tabular data, organized by Region, includes Census 2010 data and population projections for 2020 - 2070	Water User Group (WUG)	TWDB Water Planning, 2017 State Water Plan Projections Data, DRAFT http://www.twdb.state.tx.us/waterplanning/data/projections/2017/demandproj.asp	Projection Methodology – Draft Population and Municipal Water Demands, http://www.twdb.state.tx.us/waterplanning/data/projections/2017/doc/draft/methodology.pdf	Map and source development, LDC	2010 -2070
Air temperature and precipitation	Daily time series and monthly and annual normal values	Air Temperature (ºC or ºF), Precipitation (mm or inches)	National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center (NCDC) http://www.ncdc.noaa.gov/cdo-web/	NOAA Information Quality Guidelines, http://www.cio.noaa.gov/services_programs/info_quality.html	Summarize past and current weather conditions for reports	1972 - 2012
Average annual air temperature and precipitation	Spatial GIS data	Raster – 800 m resolution	PRISM Climate Group, Oregon State University, 30-arcsec NORMALS http://www.prism.oregonstate.edu/	PRISM Climate Group, Documentation FGDC Metadata http://www.prism.oregonstate.edu/docs/index.phtml	Map development	1981 -2010

Section B10: Data Management

Data Management Process

Samples are collected by field staff and delivered to the laboratory for analyses as described in Sections B1 and B2. Sampling information (e.g. site location number, date, time, sampling depth, etc.) is used to generate a unique sampling event in alphanumeric format by TWRI into a Microsoft Access database. Measurement results from the field data sheets are manually entered by field personnel into the TWRI database for their corresponding event. Data generated by the lab are entered on to the lab data sheets which are then transferred to TWRI. TWRI staff will enter these lab data into their database for the corresponding event. Customized data entry forms facilitate accurate data entry. Following data verification and validation by the TWRI Data Manager, the data are exported from the TWRI database into the pipe delimited Event/Result format required for submission to TCEQ's SWQMIS (as described in the SWQM DMRG (TCEQ 2013) or later version). Once TSSWCB and TCEQ approval of the data is obtained, the data are loaded into SWQMIS by TCEQ data managers.

The TWRI Project Manager/QAO/Data Manager is responsible for electronically transmitting the data to the TCEQ Data Management and Analysis Team for upload to SWQMIS. A completed Data Summary, as described in the most recent version of *TCEQ SWQM Data Management Reference Guide* (TCEQ 2013), will be submitted with each data submittal. If errors are found after the TCEQ review, those errors are corrected by the TWRI Project Manager/QAO/Data Manager, logged in a data correction log and all participants are notified.

This process is outlined in Figure B10-1.

Personnel

Dr. Kevin Wagner is the TWRI PM and will provide overall project management for TWRI. He is responsible for ensuring that the data are managed according to the data management plan and QAPP.

Mr. Lucas Gregory is the TWRI Principal Investigator and QAO is responsible for ensuring that project data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the data quality objectives of the project, and are reportable to TSSWCB.

Mr. Matt Brown is a TWRI Program Coordinator, Data Manager and Field Coordinator and is responsible for ensuring the use of appropriate data collection techniques in the field, it's proper documentation on field data sheets and the timely delivery of samples to the appropriate lab. He is also responsible for data storage, processing and delivery to TSSWCB.

Hardware and Software Requirements

Hardware configurations are sufficient to run Microsoft Access 2010 or newer under the Windows 7 or newer operating system in a networked environment. Information Technology (IT) staff are responsible for assuring hardware configurations meet the requirements for running current and future data management/database software as well as providing technical

support. Software development and database administration are also the responsibility of the IT department.

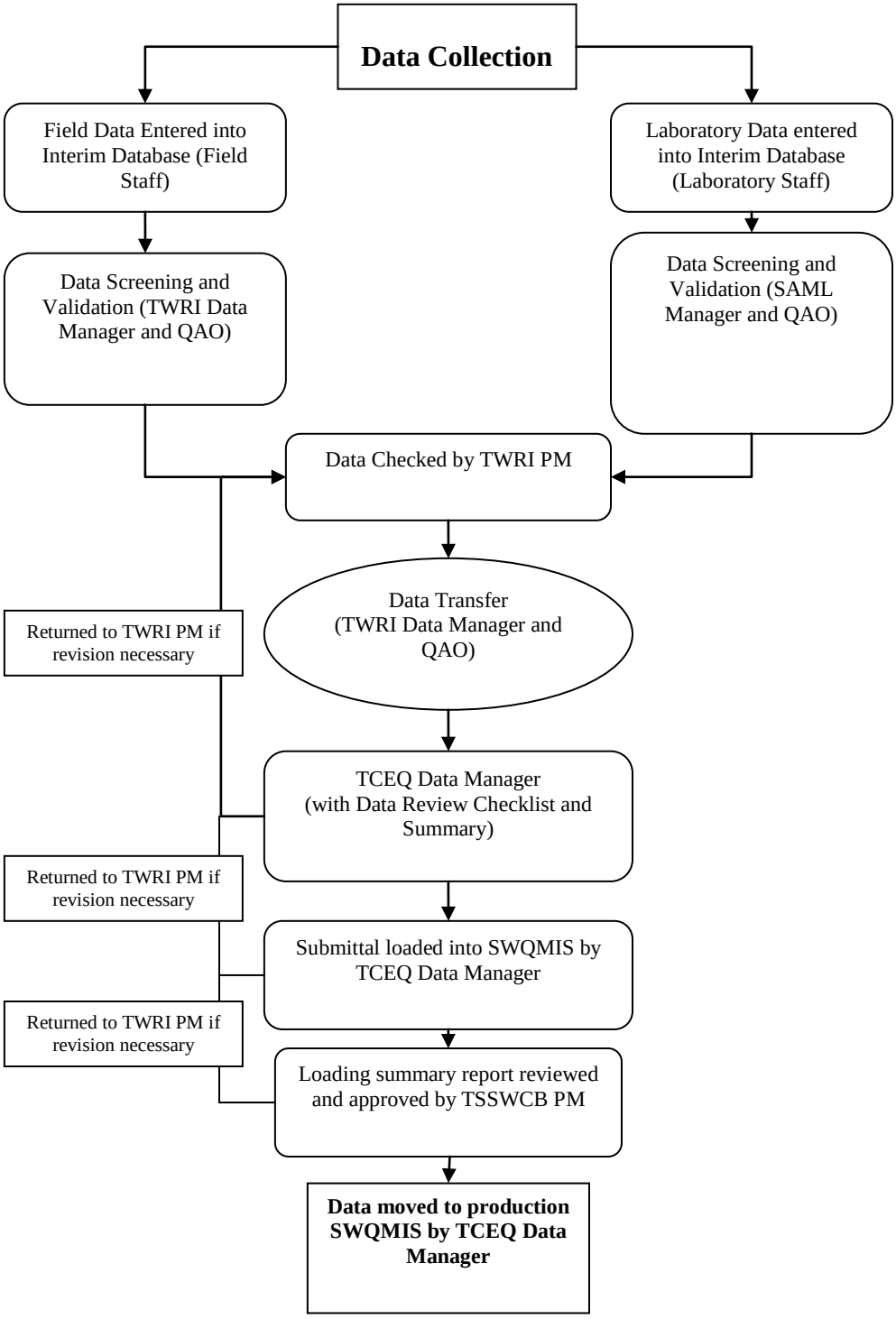


Figure B10-1. Data Management Processing Flow Chart

The types of TWRI computer equipment, hardware, and software to be used on the project are provided below in Table B10-1. Data for this project will submitted to TSSWCB using Excel workbooks, Word documents, PowerPoint presentations, and GIS files both in a format.

Table B10-1. Listing of Project Hardware and Software

Equipment & software name	Type	Number	Specification	Use
Dell or Lenovo PC Computers	Hardware	2	P4, CPU 3.2 GHz, 2 GB Ram, Windows 7 professional 2007 or higher	Support data gathering, data analysis, and report generation.
HP Proliant DL 180 G6 Server	Hardware	1	Intel Xeon CPU 3.0GHz,1GB RAM Windows Server	Primary Server
HP Proliant DL 180 G6 Server	Hardware	1	Intel Xeon CPU 3.0GHz,1GB RAM Windows Server	Secondary Server
ArcGIS 10.1 or higher	Software	1	Window interface	Development of maps and spatial analyses
IBM SPSS 21 or higher	Software	1	Window interface	Creation of historical bacteria database; statistical tests on seasonality
Microsoft Office 2010 Software (Excel, Word, PowerPoint)	Software	3	Windows platform	Data preparation, report writing, presentations

Data Handling

Data are processed using the Microsoft Access 2010 or newer suite of tools and applications. Data integrity is maintained by the implementation of password protections which control access to the database and by limiting update rights to a select user group. No data from external sources are maintained in the database. The database administrator is responsible for assigning user rights and assuring database integrity.

Data Dictionary

Terminology and field descriptions are included in the SWQM DMRG (2012 or most recent version). For the purposes of verifying which entity codes are included in this QAPP, Table 10-2 outlining the entity codes that will be used when submitting data under this QAPP is included below:

Table B10-2. SWQMIS Entity Codes Used

Sample Description	Tag Prefix	Submitting Entity	Collecting Entity	Monitoring Type
Routine monitoring to establish baseline conditions	*TX*	*TX*	*WR*	*RTLF*

Migration/Transfer/Conversion
The TWRI Data Manager is responsible for the oversight of the transfer of electronic data files from the Internet to the project directory, which is located on the TWRI Intranet. The various types of data to be downloaded from the Internet are included in Table B9.1. Data produced through this project will also be stored as a digital copy. Copies will be saved to the project directory. Transfer of water quality data to TSSWCB will occur via email attachments or uploaded to a TWRI file sharing website for retrieval by TSSWCB upon request.

Databases on the Internet are stored in a variety of formats. Some data or files required for the project can be downloaded from the Internet into text or Excel files, where they can be manipulated to create text files or other types of data files that can be used directly by models. TCEQ SWQMIS water quality data is downloaded into Excel for ease of manipulation and processing.

Backup/Disaster Recovery
TWRI utilizes Texas A&M AgriLife Research Procedure 29.01.99A0.02, Enterprise File Service (EFS). This procedure establishes enterprise file services standard operating procedures for all of Texas AgriLife Research positions and outline features and service levels, and to establish formal guidelines and procedures related to the use of the service. These procedures are established to achieve the following:

- To ensure compliance with applicable statutes, regulations, and rules regarding data retention and management;
- To define required practices regarding the use of enterprise file services;
- To educate individuals who may use enterprise file services with respect to their responsibilities associated with such use

A full copy of the EFS procedure can be found at:
http://agrilifeas.tamu.edu/documents/290199a002.pdf

Archives/Data Retention
Complete original data sets are archived on permanent paper and electronic media and retained on-site by TWRI for a retention period specified in section A9.

Record-keeping and Data Storage
TWRI record keeping and document control procedures are contained in the water quality sampling and SOPs and this QAPP. Original field and laboratory data sheets are stored in the TWRI offices in accordance with the record-retention schedule in Section A9. Electronic copies of the data sheets are also maintained on network servers, external drives and personal computers. The database backed up following each data entry event on network servers, external drives and personal computers. If necessary, disaster recovery will be accomplished by information resources staff using the backup database.

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

Forms and Checklists
See Appendices B and F for the Field and Laboratory Data Sheets. See Appendix D for the Data Review Checklist and Summary.

Information Dissemination
Project updates will be provided to the TSSWCB PM in progress reports and the information will be made available at stakeholder meetings as appropriate. Environmental data collected as part of the project described in this QAPP will be accessible to the general public from the TCEQ SWQMIS database once the data has undergone the QA/QC protocol described.

Section C1: Assessments and Response Actions

SWQM and BST

Table C1-1 presents the types of assessments and response action for activities applicable to this QAPP.

Table C1-1. Assessments and Response Actions

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TWRI	Monitor project status and records to ensure requirements are being fulfilled. Monitoring & review performance & data quality	Report to TSSWCB in QPR.
Equipment testing	As needed	SAML/TWRI/ NAWA	Pass/Fail equipment testing	Repair or replace
Data completeness	As needed	SAML/TWRI/ NAWA	Assess samples analyzed vs. planned analysis	Reanalyze or amend objectives
Laboratory Inspections	TBD by TSSWCB	TSSWCB	Analytical and QC procedures in the laboratory	30 days to respond to TSSWCB with corrective actions
Technical systems audit	As needed	TSSWCB	Assess compliance with QAPP; review facility and data management as they relate to the project	30 days to respond to TSSWCB with corrective actions
Monitoring Systems Audit	Once per life of project	TSSWCB	Assess compliance with QAPP; review field sampling and data management as they relate to the project	30 days to respond to TSSWCB with corrective actions

Deficiencies and Corrective Action

Deficiencies are any deviation from the QAPP, SWQM Procedures Manual, SOPs, or DMRG. Deficiencies may invalidate resulting data and may require corrective action. Corrective action may include for samples to be discarded and re-collected. Deficiencies are documented in logbooks, field data sheets, etc. by field or laboratory staff. It is the responsibility of the TWRI PM, in consultation with the TWRI QAO, to ensure that the actions and resolutions to the problems are documented and that records are maintained in accordance with this QAPP. In addition, these actions and resolutions will be conveyed to the TSSWCB PM in writing in the project progress reports and by completion of a CAR.

Corrective Action Reports (CARs) should:

- Identify the problem, nonconformity, or undesirable situation
- Identify immediate remedial actions if possible
- Identify the underlying cause(s) of the problem
- Identify whether the problem is likely to recur, or occur in other areas

- Evaluate the need for Corrective Action
- Use problem-solving techniques to verify causes, determine solution, and develop a CAR
- Identify personnel responsible for action
- Establish timelines and provide a schedule
- Document the corrective action in a CAR

Status of CARs will be included with quarterly progress reports. In addition, significant conditions (i.e., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported to the TSSWCB immediately.

The TWRI PM is responsible for implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the TWRI PM and/or Quality Assurance Officer. Corrective action documentation will be submitted to the TSSWCB PM with the progress report.

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

LDC and GIS Inventory

In addition to those listed above, the following assessment and response actions will be applied to LDC and GIS activities. As described in Section B9 (Non-direct Measurements), modeling staff will evaluate data to be used LDC and GIS assessments according to criteria discussed in Section A7 (Quality Objectives and Criteria for Model Inputs/Outputs Data) and will follow-up with the various data sources on any concerns that may arise.

Corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problem and will be documented utilizing CARs. CARs (Appendix A) will be filled out to document the problems and the remedial action taken. Copies of CARs will be included in QPRs and will discuss any problems encountered and solutions made. These CARs are the responsibility of the QAO and the PM and will be disseminated to individuals listed in section A3.

Section C2: Reports to Management

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

If the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problem. CARs will be filled out to document the problems and the remedial action taken. Copies of CARs will be included in quarterly progress reports.

The final report for this project will be the Navasota River WPP and will include technical information detailing the results of BST analysis, GIS assessments, LDCs and SWQM work conducted under this QAPP. Items in this report will include a very brief description of methodologies utilized and implications of these findings.

Section D1: Data Review, Validation and Verification

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

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

Section D2: Validation Methods

SWQM

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

Data review, verification, and validation will be performed using self-assessments and peer and management review as appropriate to the project task. The data review tasks to be performed by field and laboratory staffs are listed in the first column of Table D2-1. Potential errors are identified by examination of documentation and by manual *(or computer-assisted)* examination of corollary or unreasonable data. If a question arises or an error is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented. If an issue cannot be corrected, the task manager consults with the higher level project management to establish the appropriate course of action, or the data associated with the issue are rejected and not reported to the TSSWCB for submission to TCEQ for storage in SWQMIS. Field and laboratory reviews, verifications, and validations are documented.

After the field and laboratory data are reviewed, another level of review is performed once the data are combined into a data set. This review step as specified in Table D2-1 is performed by the TWRI Data Manager and QAO. Data review, verification, and validation tasks to be performed on the data set include, but are not limited to, the confirmation of laboratory and field data review, evaluation of field QC results, additional evaluation of anomalies and outliers, analysis of sampling and analytical gaps, and confirmation that all parameters and sampling sites are included in the QAPP.

The Data Review Checklist (See Appendix D) covers three main types of review: data format and structure, data quality review, and documentation review. The Data Review Checklist is transferred with the water quality data submitted to the TSSWCB to ensure that the review process is being performed.

Another element of the data validation process is consideration of any findings identified during the monitoring systems audit conducted by the TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. After the data are reviewed and documented, the TWRI PM validates that the data meet the data quality objectives of the project and are suitable for reporting to TSSWCB and subsequently TCEQ.

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

LDC
There is no validation and calibration for LDCs as they are developed using data processors.

Water quality and streamflow data collected by the TCEQ, the USGS, and Texas CRP partners have been verified and validated according to the requirements of the respective programs prior to their use in this project. Data compilations created for this project will be visually screened for errors by TWRI Staff. To verify the correctness of FDCs/LDCs, the TWRI Staff will ensure that the methods for the development of FDCs/LDCs (USEPA 2008) are followed and will verify that data formatting and inputting were done correctly and that outputs were produced error free.

GIS Inventory
Data for this portion of the project (e.g., land use, urban areas, population projections, digital elevation models, stream layers, and population projections) as provided in Table B9.1 have been collected and made publicly accessible by authoritative sources such as the USGS, USDA, USEPA, and U.S. Census Bureau. Data from these sources will be considered as verified and validated by the various agencies providing the data. However, data compilations created for this project will be visually screened for errors. Any errors detected by project staff will be reported to the TWRI PM and, if necessary, to the TSSWCB PM for resolution. Issues which can be readily corrected, e.g., removal of outlier data, will be documented and the data either removed or corrected prior to further analysis.

BST
In addition to the verification steps outlined for SWQM, the SCSC Project Lead, with assistance from the TWRI PM/QAO, is responsible for validating that the verified BST data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data for anomalies. The SCSC Project Lead may designate other experienced water quality experts (TWRI PM or Field Supervisor) familiar with the waterbodies under investigation to perform this evaluation. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data before data validation can be completed.

A second element of the validation process is consideration of any findings identified during the monitoring systems audit conducted by the TWRI PM/QAO or TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the TWRI PM/QAO validates that the data meet the DQOs of the project and are suitable for reporting to the TSSWCB.

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

Data to be Verified	Field† Supervisor	Laboratory Supervisor	PM/QAO Task‡
Collection & analysis techniques consistent with SOPs & QAPP	X	X	X
Field QC samples collected for all parameters as prescribed in the QAPP	X		X
Field documentation complete	X		X
Instrument calibration data complete	X	X	X
Sample documentation complete	X	X	X
Field QC results within acceptance limits	X		X
Sample identifications	X	X	X
Chain of custody complete/acceptable	X	X	X
Sample preservation and handling	X	X	X
Holding times	X	X	X
Instrument calibration data	X	X	X
QC samples analyzed at required frequencies		X	X
QC samples within acceptance limits		X	X
Instrument readings/printouts	X	X	X
Calculations	X	X	X
Laboratory data verification for integrity, precision, accuracy, and validation		X	X
Laboratory data reports		X	X
Data entered in required format	X	X	X
Site ID number assigned	X		X
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
GIS data			X

† Field and Laboratory Supervisor may be the same person

‡ TSSWCB PM / QAO will monitor data for QA/QC purposes as needed.

All other entities are required to inspect 100% of the data prior to approval

Section D3: Reconciliation with User Requirements

SWQM

Data produced in this project, and data collected by other organizations will be analyzed and used in the development water quality restoration plans. Data that do not meet requirements described in this QAPP will not be submitted to SWQMIS nor will it be considered appropriate for any of the uses noted above.

Data collected from this project will be analyzed by TWRI to document the current state of water quality in Navasota River. Data will be used to augment the existing load duration curve for the monitored site and geometric means will be compared to the water quality standard.

Data produced in this project will be analyzed and reconciled with project data quality requirements. Data meeting project requirements may be used by the TCEQ for the *Texas Water Quality Integrated Report* in accordance with TCEQ's *Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data* (TCEQ 2010), and for TMDL development, water quality standards development, and permit decisions as appropriate. Data that do not meet data quality objectives outlined in this document will not be submitted to SWQMIS.

LDC

The LDC framework utilized for this project will be used to determine maximum allowed bacteria (*E. coli*) loadings within the water bodies evaluated in the Navasota River Basin. This approach will utilize historical flow data and the primary contact recreation criterion for waters to determine this pollutant load allocation. Exceedances of the allowable load for each waterbody will be determined using the procedures outlined in USEPA (2008) by the TWRI Staff and will provide the basis for future load reductions needed.

The LDC results will be described in detail in the final report and used for educational purposes as appropriate and will aid in making informed decisions about future action to address pollutant loading issues across the watershed. The limitations of LDCs produced will also be described in the report and conveyed to audiences when discussed.

GIS Inventory

GIS inventory and maps developed for this project will be used for informational purposes only and will not be used exclusively to make any management decisions. Instead, these maps will aid the user by allowing them to visualize watershed features and influences within the watershed that could contribute to the overall bacteria loading being experienced. The limitations of maps produced will be described in the project final report and conveyed to audiences when discussed. Potential limitations may include accuracy and precision of the land use data, planning documents, and societal information used.

BST

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

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

References

Bernhard, A.E. and K.G. Field. 2000. "A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA." Appl Environ Microbiol. 66(10): 4571-4574.

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

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

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

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

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

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

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

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

TCEQ. 2014. Surface Water Quality Monitoring Data Management Reference Guide. November 2014. http://www.tceq.state.tx.us/compliance/monitoring/water/quality/data/wdma/dmrg_index.html

TCEQ. 2013. Surface Water Quality Data Management Reference Guide. Water Quality Planning Division, Monitoring & Assessment Section, Data Management & Analysis Team. http://www.tceq.texas.gov/waterquality/data-management/dmrg_index. html.

TCEQ. 2012a. 2012 Guidance for Assessing and Reporting Surface Water Quality in Texas (May 2012): In Compliance with Sections 305(b) and 303(d) of the Federal Clean Water Act.

TCEQ. 2012b. SWQM Procedures, Volume 1: Physical and Chemical Monitoring Methods. TCEQ, RG-415 Revised August 2012.

TCEQ. 2010. Guidance for Screening and Assessing Texas Surface and Finished Drinking Water Quality Data (August 25, 2010).

http://www.tceq.texas.gov/assets/public/compliance/monops/water/10twqi/2010_guidance.pdf

TCEQ. 2010. OPP 8.11: Geographic Information Systems – Spatial Data, October 2010.

TCEQ. 2010. OPP 8.12: Global Positioning System, October 2010.

TCEQ. 2007. SWQM Procedures, Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data. TCEQ, RG-416.

The NELAC Institute (TNI) Standard. 2009. Volume 1, Management and Technical Requirements for Laboratories Performing Environmental Analysis, Module 2, Quality Systems General Requirements. EL-V1M2-ISO-2009.

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

USEPA. 2001. EPA Requirements for Quality Assurance Project Plans. EPA QA/R-5. http://epa.gov/quality/qatools.html. Requirements. EL-V1M2-ISO-2009.

Zeckoski, R.W., B.L. Benham, S.B. Shan, M.L. Wolfe, K.M. Brannan, M. Al-Smadi, T.A. Dillaha, S. Mostaghimi, and C.D. Heatwole, 2005. BSLC: A tool for bacteria source characterization for watershed management. Transactions of ASAE, 21(5): 879-889.

This Page Left Blank Intentionally

Appendix A: Corrective Action Report

SOP-QA-001
CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

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

Possible causes:
_____
_____
_____

Recommended Corrective Actions:
_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:
_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Program Manager:_____

TWRI Quality Assurance Officer:_____

TSSWCB Quality Assurance Officer:_____

Appendix B: Field Data Reporting Form

Navasota River Water Quality Monitoring Field Data Form

PLEASE PRINT (Black, Indelible Ink)

Monitor's Name: Station ID #:

Sample Location: Sample Type: Routine

Date: (M M D D Y Y) Sample Time (24 hr): (H H M M) Sample Depth (Meters): (Not Total Depth)

Field Measurements (Gray Boxes for Stream Team (Recon) Sampling; in addition to white boxes)

Code	Data	Descriptor
00400		ph (Standard Units)
00010		Water Temperature (Celsius)
00300		Dissolved Oxygen (mg/L)
00094		Specific Conductance (micro S/cm)
00061		Instant. Stream Flow (cfs)
89835		Flow Meth 1=Gage, 2=Elec, 3=Mech, 4=Weir/Flu, 5=Doppl
01351		Flow Severity 1=No Flow, 2=Low, 3=Normal, 4=Flood, 5=High, 6=Dry
00076		Turbidity (FTU)
89966		Present Weather 1=Clear, 2=Prt. Cloudy, 3=Cloudy, 4=Rain, 5=Other
72053		Days Since Last Precipitation Event
00020		Air Temperature (Celsius)
00078		Secchi Depth (Meters)
82903		Depth to water bottom at sample site (Meters)
89968		Water Surface 1=Calm, 2=Ripples, 3=Waves, 4=White Caps
82553		Rainfall in 1 day inclusive prior to sample (IN)
82554		Rainfall in 7 Days inclusive prior to sample (IN)
89978		Number of People Observed Engaging in Contact Recreation
89979		Evidence of Primary Contact Recreation (0=not observed; 1=observed)
		Water Clarity 1=Clear, 2=Cloudy, 3=Turbid
		Water Color 1=No Color, 2=Lt Grn, 3=Dk Grn, 4=Tan, 5=Red, 6=Grn/Brn, 7=Blk
		Water Surface 1=Clear, 2=Scum, 3=Foam, 4=Debris, 5=Sheen
		Water Ordor 1=None, 2=Oil, 3=Acrid, 4=Sewage, 5=Rotten Eggs, 6=Fishy, 7=Musky
		Algae Cover 1=Absent, 2=Rare, 3=Common, 4=Abundant, 5=Dominant
		Rainfall Accumulation Over Last 3 Days (inches)

Parameters Collected (Circle Appropiate): *E. coli* (IDEXX) 31699 *E. coli* (mTEC) 31648

Other Observations:

Comments:

I CERTIFY THAT ALL PROCEDURES HAVE BEEN FOLLOWED AND THIS INFORMATION IS ACCURATE TO THE BEST OF MY ABILITY.

MONITOR'S SIGNATURE DATE DATA MANAGER'S SIGNATURE DATE

Appendix C: Chain of Custody Record

Texas Water Resources Institute
1500 Research Pkwy, Ste 110, College Station, TX 77843-2260

CHAIN OF CUSTODY RECORD

Project Name: Watershed protection plan development for the Navasota River below Lake Limestone					# of containers	Name of Sampler:_____Sampler's Phone						
						Analysis Information						
Station ID	Date	Sampling Time (24hr)	Matrix	Description		E. coli (method)	Lab Sample Delivered to: SAML or NAWA	Analyzing Lab Tech	Analysis Start Time	Analysis End Time	Final Count	Sample ID
11875			Water									

Relinquished by: (Signature)	Date:	Time:	Received by: (Signature)	Date:	Time:	Laboratory remarks:
Relinquished by: (Signature)	Date:	Time:	Received by: (Signature)	Date:	Time:	Lab log #
Relinquished by: (Signature)	**Date:**	**Time:**	**Received by: (Signature)**	**Date:**	**Time:**	**Laboratory Name:**

Appendix D: Data Review Checklist

DATA REVIEW CHECKLIST

QAPP Title: _____

Effective Date of QAPP: _____

Data Format and Structure		**Y, N, or N/A**
A.	Are there any duplicate *Tag Id* numbers in the Events file?	
B.	Do the *Tag* prefixes correctly represent the entity providing the data?	
C.	Have any *Tag Id* numbers been used in previous data submissions?	
D.	Are TCEQ station location (SLOC) numbers assigned?	
E.	Are sampling *Dates* in the correct format, MM/DD/YYYY with leading zeros?	
F.	Are the sampling *Times* based on the 24 hour clock (e.g. 13:04) with leading zeros?	
G.	Is the *Comment* field filled in where appropriate (e.g. unusual occurrence, sampling problems, unrepresentative of ambient water quality)?	
H.	*Submitting Entity*, *Collecting Entity*, *and Monitoring Type* codes used correctly?	
I.	Are the sampling dates in the *Results* file the same as the one in the *Events* file for each Tag Id?	
J.	Are values represented by a valid parameter code with the correct units?	
K.	Are there any duplicate parameter codes for the same *Tag Id*?	
L.	Are there any invalid symbols in the *Greater Than/Less Than* (*GT/LT*) field?	
M.	Are there any *Tag Ids* in the *Results* file that are not in the *Events* file or vice versa?	
Data Quality Review		**Y, N, or N/A**
A.	Are all the "less-than" values reported at the LOQ? **If no, explain on next page**.	
B.	Have the outliers been verified and a "1" placed in the *Verify_flg* field?	
C.	Have checks on correctness of analysis or data reasonableness been performed?	
D.	Have at least 10% of the data in the data set been reviewed against the field and laboratory data sheets?	
E.	Are all parameter codes in the data set listed in the QAPP?	
F.	Are all stations in the data set listed in the QAPP?	
Documentation Review		**Y, N, or N/A**
A.	Are blank results acceptable as specified in the QAPP?	
B.	Were control charts used to determine the acceptability of field duplicates?	
C.	Was documentation of any unusual occurrences that may affect water quality included in the *Event* table's *Comments* field?	
D.	Were there any failures in sampling methods and/or deviations from sample design requirements that resulted in unreportable data? **If yes, explain on next page**.	
E.	Were there any failures in field and/or laboratory measurement systems that were not resolvable and resulted in unreportable data? **If yes, explain on next page.**	
F.	Was the laboratory's TNI Accreditation current for analysis conducted?	

Data Set Information

Data Source:

Date Submitted:

Tag_ID Range:

Date Range:

Comments:

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

- Inconsistencies with AWRL specifications or LOQs
- Failures in sampling methods and/or laboratory procedures that resulted in data that could not be reported to the TCEQ
- Include completed Corrective Action Reports with the applicable Progress Report

_____
_____
_____
_____
_____
_____
_____
_____
_____

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

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

TWRI Data Manager:

Date:

Appendix E: Texas BST Standard Operating Procedures

E-1: Archival of *Escherichia coli* Isolates

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Carefully seal plate using an adhesive PCR cover.

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

    a. Initial denaturation at 95°C for 10 min

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

    c. Final Extension at 72°C for 10 min

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

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

10. Allow gel to solidify for approximately 30 minutes on the bench, then without removing comb place in Ziploc bag and solidify overnight in the refrigerator. The next day carefully remove comb, transfer to gel tank in cold room (4°C) containing pre-cooled 1X TBE buffer. Replace TBE in gel tank after it has been used twice.

11. The following items will be needed for electrophoresis:

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

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

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

    150 µL 10X PCR buffer

    850 µL molecular grade water

    Store in cold room

    6X ERIC-PCR Loading Buffer

    25 mg bromphenol blue (0.25%)

    1.5 g ficoll 400 (15%)

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

    ERIC-PCR Blank

    100 µL 10X PCR buffer

    200 µL 6X ERIC-PCR loading buffer

    900 µL molecular grade water

    Store in cold room

    Ethidium Bromide Stain (0.5 µg/mL)

1250 mL 1X TBE

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

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

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

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

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

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

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

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

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

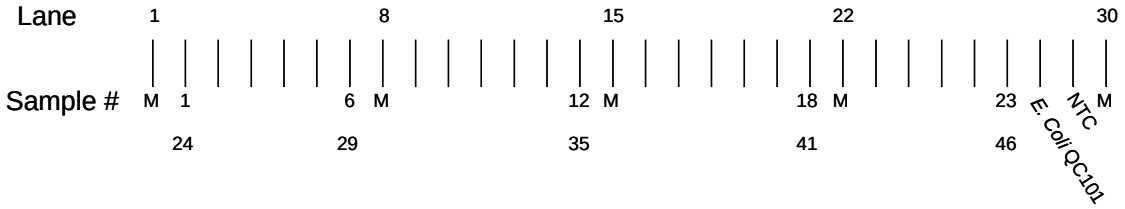
    f. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

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

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

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

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



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

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

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

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

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

18. Follow Gel Logic 200 SOP for image capture. Save digital photograph as a TIFF file (default) and print a hardcopy for notebook.

E-3: RiboPrinting of *Escherichia coli*

Storing and Handling Disposables

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

Heating membrane and probe (MP) Base

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

To degas buffer:

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

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

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

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

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

Sample Preparation Procedures

Incubate and Inspect the Samples

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

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

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

3. Add sample buffer to microcentrifuge tubes

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

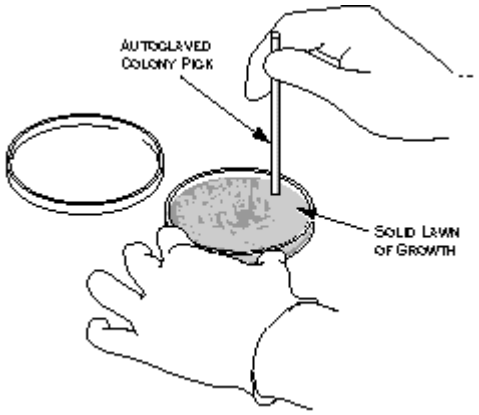
    2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.

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

    3. Close the lids on the tubes.

Harvest the Samples

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



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

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

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

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.

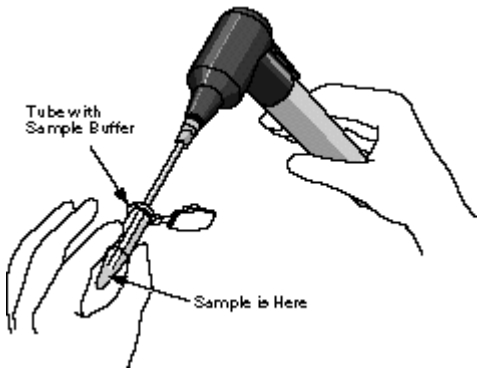
3. Press the ON lever on the mixer for about 5 seconds.

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

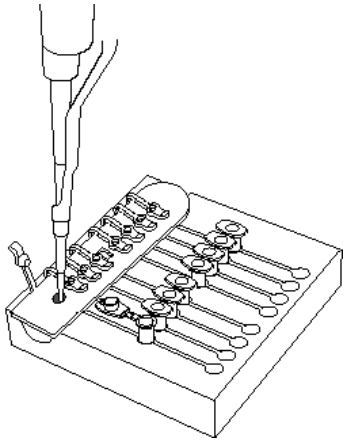
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.

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.



Transfer the Samples to the Sample Carrier



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

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

3. Close the lid cover for the well.

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

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

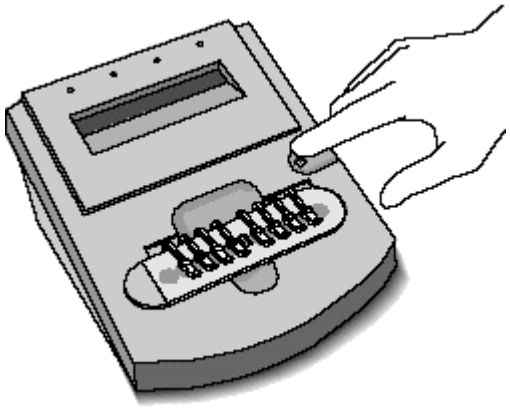
5. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).

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

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

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

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



2. Press the button on the Heat Treatment Station.

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

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

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

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

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

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

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

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

Creating and Loading a Batch

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

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

You can also create special batches:

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

From the Instrument Control Base Window:

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

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

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

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

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

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

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

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

4. 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.

5. 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.

6. 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.

7. Repeat for the other seven samples.

8. 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

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

4. 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

Remove the DNA Prep carrier from the refrigerator.

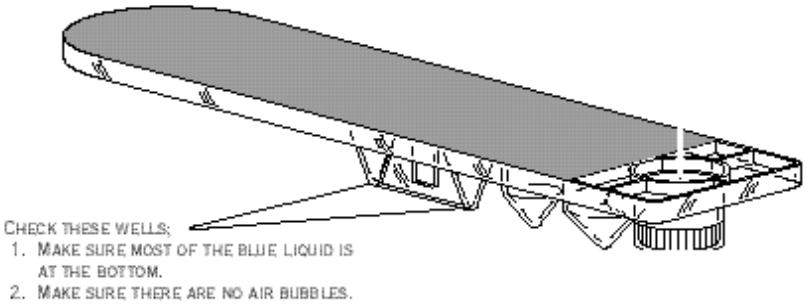
1. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**

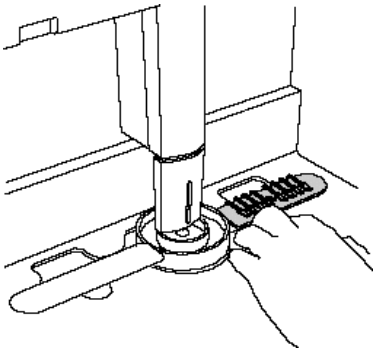
2. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.

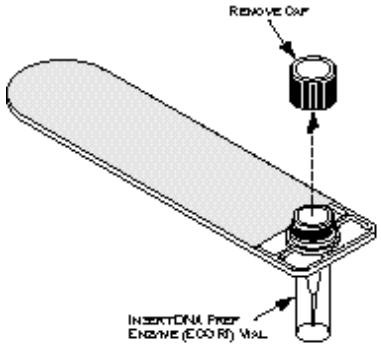
3. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as a 50 U/µL working stock as follows.**

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

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





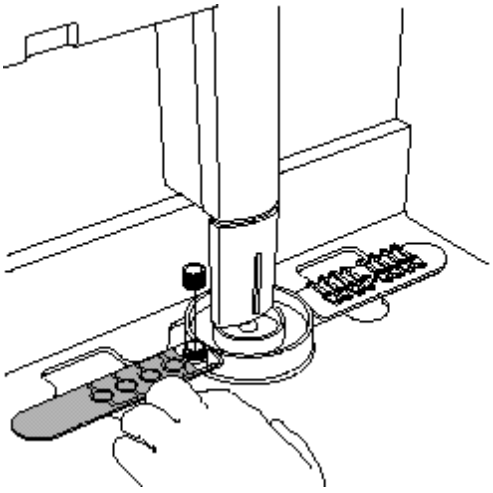


4. Remove the cap from the Enzyme vial.

5. Insert the vial into the carrier.

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

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

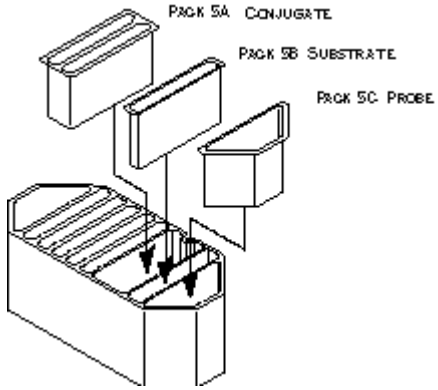


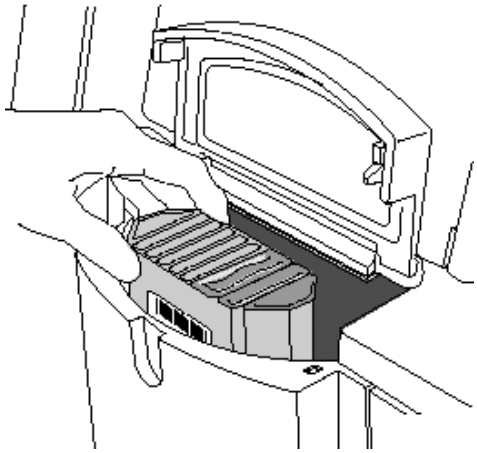
4. Load the MP Base and Carousel

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.

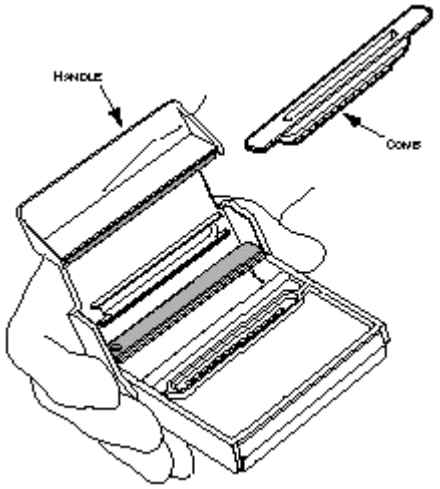
5. Load the Gel Cassette

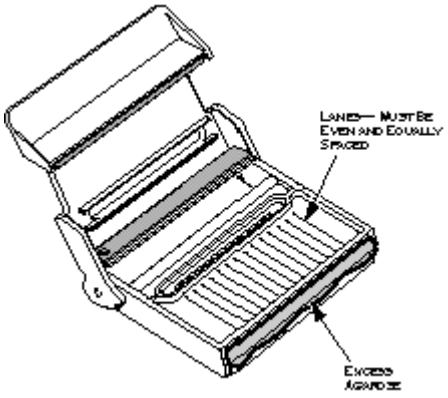
1. Remove the gel cassette from its package.

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.

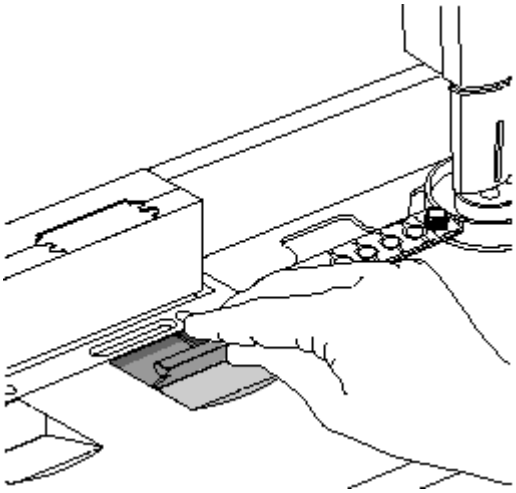
4. Check the front edge of the gel cassette and the lanes of the gel.





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.

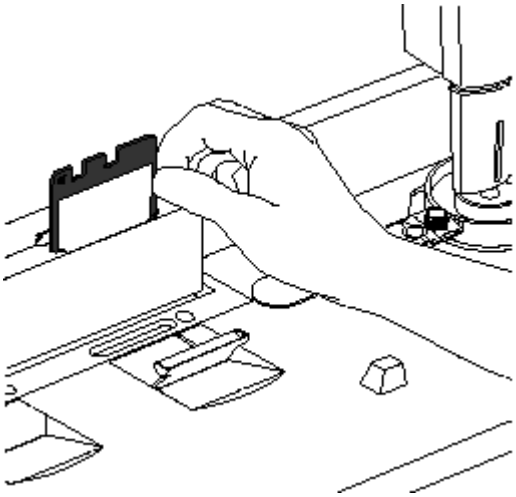


6. Press the cassette forward firmly until it snaps into place.

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

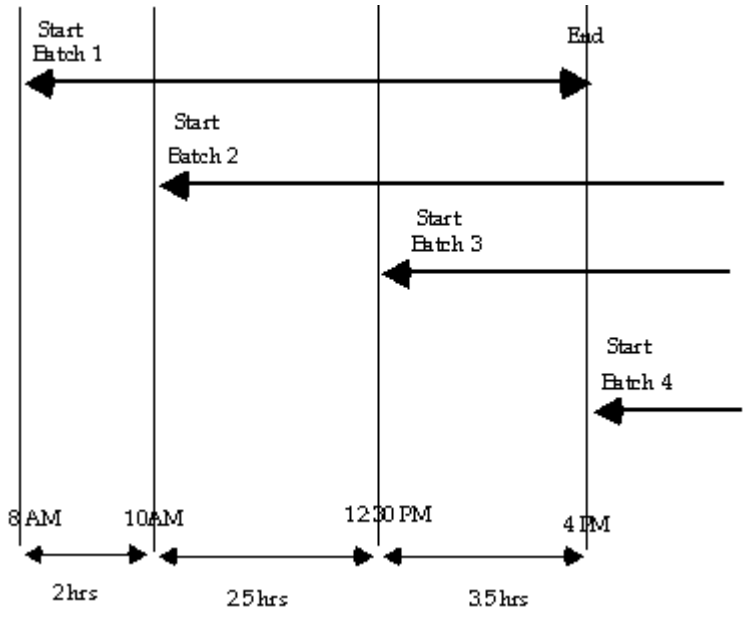
The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.
The chart above shows the approximate loading times for each batch in a work shift using only the VCA protocol.

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 F: SAML Lab Bench Sheet

SAML DATA LOG SHEET:
EPA METHOD 1603

Data Log
Revision
Effective

	Time Analyst Initials:					Volume Filtered (mls) and Counts Analyst Initials:				
	Time Filtered	IN Incubator#1 (35°C)	OUT Incubator#1 (35°C)	IN WtrBth#1 (44.5°C)	OUT WtrBth#1 (44.5°C)					
. coli										
. fecalis										
ecalis										
k (50mls)										
(50ms)										
n Tube Blank (9mls)										

Page _____

SAML DATA LOG SHEET:
EPA METHOD 1603

Data Log
Revision
Effective

nation	Media Sterility
	mTEC:
	TSA:
	BHI:
	Filter Sterility:
r:	
25ml 10ml	