Texas Nonpoint Source Grant Program

Texas Bacterial Source Tracking Program (FY24-FY25)

TSSWCB Project 24-50

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

Prepared by:

Texas Water Resources Institute
The University of Texas Health Science Center at Houston School of Public Health in El Paso
Texas A&M AgriLife Research Soil and Aquatic Microbiology Lab
Bandera County River Authority & Groundwater District

Effective Period: Upon TSSWCB Approval through January 31, 2026

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

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Section A1: Approval Sheet

Quality Assurance Project Plan (QAPP) for the *Texas Bacterial Source Tracking Program (FY24-FY25).*

Texas State Soil and Water Conservation Board (TSSWCB)

Name: Anyssa Devers
Title: TSSWCB Project Manager (PM)

Signature:_____ Date: _____

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

Signature:_____ Date: _____

Texas A&M AgriLife Research – Texas Water Resources Institute (TWRI)

Name: Lucas Gregory, PhD
Title: TWRI Associate Director; Project Lead and Quality Assurance Officer (QAO)

Signature:_____ Date: _____

Name: Amanda Tague
Title: TWRI Project Manager (PM)

Signature:_____ Date: _____

Texas A&M AgriLife Research – Soil and Aquatic Microbiology Lab (SAML)

Name: Terry Gentry, PhD
Title: Professor of Soil & Aquatic Microbiology; SAML Director & Project Co-Lead

Signature:_____ Date: _____

The University of Texas Health Science Center at Houston School of Public Health, El Paso Regional Campus (UTHealth H SPH)

Name: Anna Gitter, Ph.D.
Title: Assistant Professor; UTHealth H SPH Project Co-Lead

Signature:_____ Date: _____

Bandera County River Authority & Groundwater District (BCRAGD)

Name: Clint Carter
Title: Project Manager/QAO/Data Manager

Signature:_____ Date: _____

Section A2: Table of Contents

List of Acronyms and Abbreviations

BCRAGD	Bandera County River Authority & Groundwater District
BST	Bacterial source tracking
CAR	corrective action report
COC	chain of custody
C_T	threshold cycle
DQO	data quality objectives
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
FIB	fecal indicator bacteria
MPN	Most Probable Number
MS Excel	Microsoft Excel
mTEC	membrane thermotolerant *E. coli*
MUG	4-methylumbelliferyl-β-D-glucuronide
NA-MUG	nutrient agar with MUG
NIST	National Institute of Standards and Technology
NGS	next generation sequencing
PM	Project Manager
QA	quality assurance
QAPP	quality assurance project plan
QAO	Quality Assurance Officer
QC	quality control
QMRA	quantitative microbial risk assessment
QPR	quarterly progress report
RPD	Relative percent deviation
SAML	Texas A&M AgriLife Research- Soil and Aquatic Microbiology Lab
SOP	Standard operating procedure
spp	species
TCEQ	Texas Commission on Environmental Quality
TIGSS	Texas A&M Institute for Genome Sciences and Society
TMDL	total maximum daily load
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas A&M AgriLife Research, Texas Water Resources Institute
USEPA	United States Environmental Protection Agency
UTHealth H SPH	University of Texas Health Science Center at Houston School of Public Health in El Paso
UV	ultraviolet
WPP	Watershed protection plan

Section A3: Distribution List

Organizations, and individuals within, which will receive copies of the approved quality assurance project plan (QAPP) and any subsequent revisions include:

Texas State Soil and Water Conservation Board
1497 Country View Lane; Temple, Texas 76504

Name: Anyssa Devers
Title: TSSWCB PM

Name: Mitch Conine
Title: TSSWCB QAO

Texas A&M AgriLife Research, Texas Water Resources Institute
2118 TAMU; College Station, TX 77840-2118

Name: Lucas Gregory, PhD
Title: TWRI Associate Director; Project Lead and QAO

Name: Amanda Tague
Title: TWRI PM

The University of Texas Health Science Center at Houston School of Public Health in El Paso (UTHealth H SPH)
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Name: Terry Gentry, PhD
Title: Professor of Soil & Aquatic Microbiology; Director & Project Co-Lead

Bandera County River Authority & Groundwater District (BCRAGD)
PO Box 177, Bandera, TX 78003

Name: Clint Carter
Title: Project Manager, Quality Assurance Officer, and Data Manager

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.

Anyssa Devers, 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 assurance (QA), quality control (QC), and reporting under the Texas Nonpoint Source Program.

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

Lucas Gregory, TWRI Associate Director and Project Lead

Responsible for supporting the development and ensuring the timely delivery of project deliverables, ensuring cooperation between project partners, providing fiscal oversight and completing project reporting.

Responsible for following the QAPP and conducting the tasks appropriately for field collection of environmental samples.

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.

Amanda Tague, TWRI PM

The TWRI Project Manager 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.

UTHealth H SPH – The University of Texas Health Science Center at Houston School of Public Health in El Paso (UTHealth H SPH), El Paso, Texas. Responsible for bacterial source tracking and health risk analyses using quantitative microbial risk assessment (QMRA).

Anna Gitter, Assistant Professor and UTHealth H SPH Project Co-Lead

Responsible for performing bacterial source tracking (BST) and human health risk analysis and related activities. This includes ensuring that laboratory and study personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and all standard operating procedures (SOP's) specific to analyses or task performed. Responsible for oversight of laboratory operations ensuring that QA/QC requirements are met, documentation related to analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective action is implemented, documented, reported and verified. Monitors implementation of measures in the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

SAML – Texas A&M AgriLife Research – Soil and Aquatic Microbiology Lab, College Station, Texas. Responsible for bacterial source tracking.

Terry Gentry, Professor of Soil & Aquatic Micro.; SAML Director & Project Co-Lead

Responsible for BST analysis and related activities. This includes ensuring that SAML personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and SOPs specific to analyses or task performed. Responsible for oversight of all SAML 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 action is implemented, documented, reported and verified. Monitors implementation of measures within SAML to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

BCRAGD – Bandera County River Authority & Groundwater District, Bandera, Texas. Responsible for bacterial source and water sample collection in the Medina River above Medina Lake watershed.

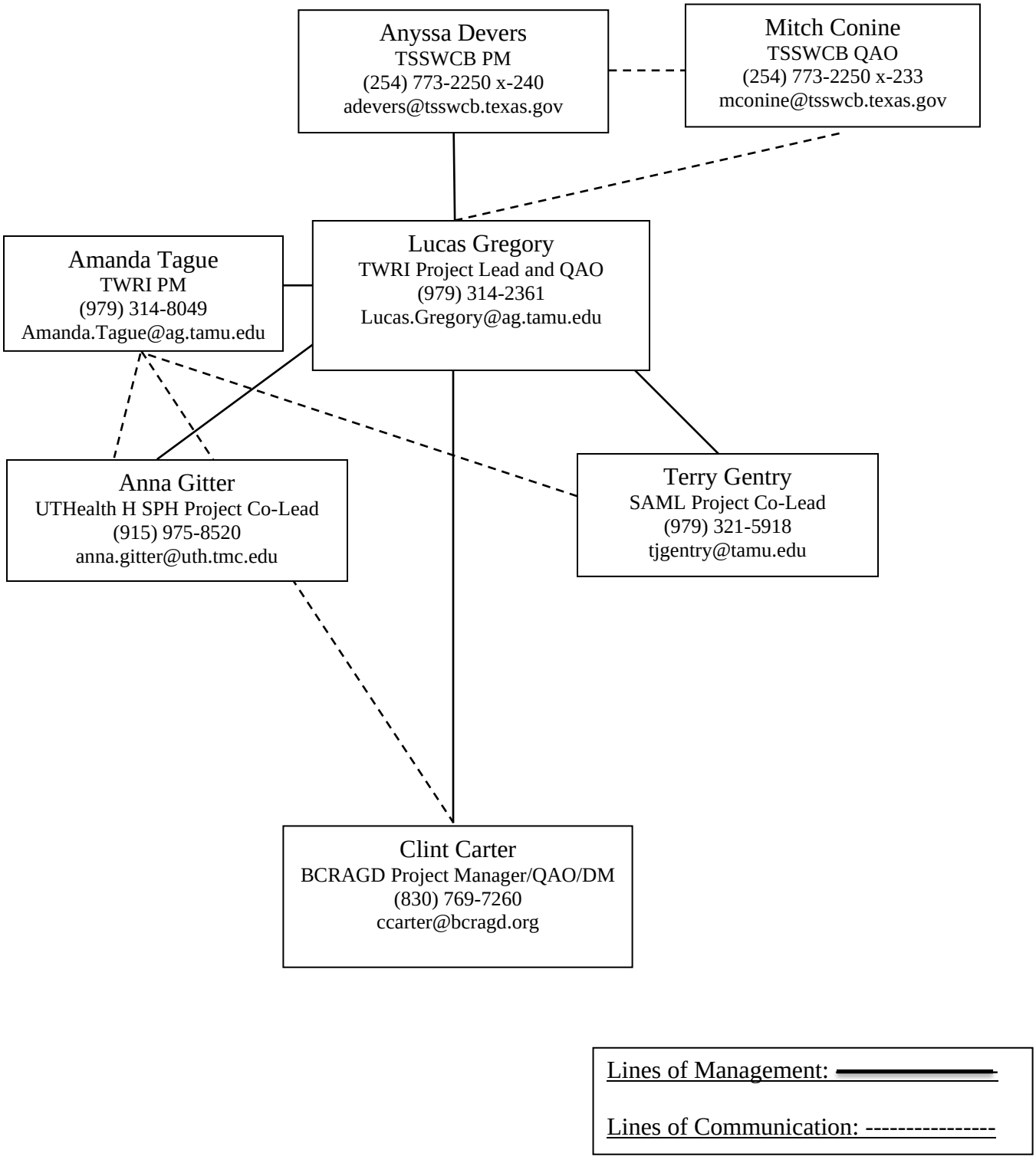
Clint Carter, Project Manager, QAO and Data Manager

Responsible for water and bacteria source sample collection, preservation, storage and delivery. Responsible for verifying the QAPP is followed and the project is producing data of known and acceptable quality. Ensures adequate training and supervision of all monitoring and data collection activities. Complies with corrective action requirements.

Responsible for ensuring that BCRAGD QA/QC requirements are met, documentation related to analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective action is implemented, documented,

reported and verified. Responsible for contributing to QAPP development. Responsible for identifying, receiving, and maintaining project quality assurance records. Responsible for coordinating with the TWRI Project Manager to resolve QA-related issues. Notifies the TWRI Project Manager and documents particular circumstances which may adversely affect the quality of data. Responsible for validation and verification of all data collected and acquired.

Figure A4.1 Project Organization Chart



Section A5: Problem Definition/Background

Bacteria continues to remain the number one cause of water quality impairments in the state of Texas. Numerous approaches have been applied to evaluate bacteria sources in streams and rivers to develop effective watershed management practices. Evaluating water quality integrity for contact recreation (and shellfish harvesting) has been dependent on measuring fecal indicator bacteria (FIB), specifically *Escherichia coli* (*E. coli*) and *Enterococcus* species. Bacteria source tracking (BST) has been identified as a valuable tool for identifying the different sources of fecal pollution, therefore informing the development of watershed plans, total maximum daily loads (TMDL) and other strategies for addressing the impairments. Comprehensive BST has been completed by UTHealth H SPH and AgriLife SCSC in numerous watersheds throughout Texas with support provided by the TSSWCB. As a result of these joint efforts over the last decade, the Texas *E. coli* BST Library (ver. 03-20) currently contains 1,942 *E. coli* isolates obtained from 1,775 different domestic sewage, wildlife, livestock, and pet fecal samples.

While comprehensive BST projects have been completed in watersheds across Texas and provide considerable value to planners working to prioritize implementation, methodological limitations exist for traditional library-dependent BST. The use of FIB has been integral in assessing exposure risks for fecal pollution, but as further research has indicated, there are limitations to solely relying on these indicators. Measuring for *E. coli* and *Enterococcus* species in recreational and shellfish harvesting waters remains the primary approach for assessing bacteria contamination and health risks in water bodies; however, advances in molecular technology with next generation sequencing (NGS) provides another tool to assess the presence of various fecal contaminants in a water body.

Early applications of NGS were limited by the lack of taxonomic resolution of pathogens due to short deoxyribonucleic acid (DNA) read lengths (approximately 60 base pairs). In recent years, sequence read lengths have continued to increase, therefore providing increased confidence in the classification of NGS-sequences to the bacterial species level (Tan et al., 2015). Further work applying NGS techniques to evaluate bacterial communities impacted by different land uses and water quality has indicated that the genera and species of pathogen sequences has varied according to land use and FIB concentrations (Nshimyimana et al., 2015).

NGS techniques have been used to assess the biodiversity of aquatic habitats, but more recently, used in water microbiology to supplement water quality monitoring efforts. These techniques provide the opportunity to simultaneously test for the presence of various pathogenic targets (e.g., bacteria, protozoa, viruses) without the need to culture specific organisms in the lab (Hamner et al., 2019; Ji et al., 2020). In addition, some newer NGS sequencing platforms are field-portable and capable of generating near real-time results thus opening possible applications for source identification in water bodies. Multiple studies have demonstrated the potential for NGS-based approaches to be used for BST and help provide a deeper understanding of the fecal sources impacting a water body (Raza et al., 2021; Unno et al., 2018). In a brief overview, NGS methods involve four key steps that include DNA isolation from the environmental sample, library preparation, sequencing, and bioinformatic data analysis. This approach is not limited by requiring a pre-selected list of microbes that require being identified by traditional culture-based, immunoassay, microscopy, or polymerase chain reaction (PCR) based analyses (Miller et al.,

2013). NGS techniques permit the DNA sequence-based characterization of a wide array of microorganisms that may be present in a water body (Hamner et al., 2019).

QMRA is a valuable tool that can integrate BST results and estimate potential human health risks in recreational waters. Using BST data and QMRA is supported by the U.S. Environmental Protection Agency's recommendation in the revised 2012 Recreational Water Quality Criteria to assess water quality based on health risks (USEPA, 2012). Efforts to delineate QMRA outputs to inform policy and best management practices can increase the utility of BST work in Texas. Finally, continued outreach and technology transfer is needed to expand awareness and understanding of BST, foster dialogue and collaboration, and bring water resource managers up to speed on advances in BST technologies, methodologies, applications, and results.

The ability to screen water samples for genetic sequences relating to waterborne pathogens assists in identifying potential human health risks and provides a preliminary characterization and distribution of pathogens in water bodies influenced by different pollutant sources. Current efforts to measure water quality and exposure risks using FIB requires inferring about potential sources of fecal pollution and if pathogens may exist. Advances in NGS methods provides the opportunity to analyze for a wide array of pathogens that has not been previously possible with traditional microbial techniques. Utilizing NGS to characterize for microbial pathogens instead of relying on FIB enumeration provides a direct identification of microorganisms that could be a risk to human health. Such information is imperative for watershed managers striving to identify management practices that reduce human exposure and therefore the health risk, to pathogens in recreational waters. Further, direct detection of pathogens can potentially prioritize sites for targeted management, therefore implementing funds and efforts that may provide the greatest protection for public health.

Advances in NGS methods provides the opportunity to further evaluate and expand the Texas BST Library, as well survey water quality for potential pathogens. Findings from this work can be utilized to evaluate the appropriateness of NGS techniques for water quality management. Continued support of the Texas BST Infrastructure project is imperative for watershed managers striving to identify management practices that reduce pollutant sources and minimize human health risks in Texas water bodies. Continued BST application across Texas will inform and guide watershed stakeholders in watershed planning and implementation efforts. Other engagement activity regarding bacteria sources and feasible management efforts is also needed to promote and support watershed protection plan (WPP) implementation.

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Ji, P., Aw, T. G., Van Bonn, W., & Rose, J. B. 2020. Evaluation of a portable nanopore-based sequencer for detection of viruses in water. *Journal of Virological Methods*, *278*, 113805.

Hamner, S., Brown, B. L., Hasan, N. A., Franklin, M. J., Doyle, J., Eggers, M. J., Colwell, R.R., & Ford, T. E. 2019. Metagenomic profiling of microbial pathogens in the little Bighorn river, Montana. *International Journal of Environmental Research and Public Health*, *16*(7), 1097.

Miller, R. R., Montoya, V., Gardy, J. L., Patrick, D. M., & Tang, P. 2013. Metagenomics for pathogen detection in public health. *Genome medicine*, *5*(9), 81.

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Raza, S., J. Kim, M.J. Sadowsky, & T. Unno. 2021. Microbial source tracking using metagenomics and other new technologies. *Journal of Microbiology, 59*, 259-269.

Tan, B., Ng, C. M., Nshimyimana, J. P., Loh, L. L., Gin, K. Y. H., & Thompson, J. R. 2015. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. *Frontiers in microbiology*, *6*, 1027.

Unno, T., C. Staley, C.M. Brown, D. Han, M.J. Sadowsky, and H.-G., Hur. 2018. Fecal pollution: new trends and challenges in microbial source tracking using next-generation sequencing. *Environmental Microbiology, 20*, 3132-3140.

U.S.EPA. 2012. Recreational Water Quality Criteria. Office of Water, United States Environmental Protection Agency: Washington, D.C., USA.

Section A6: Project/Task Description

Continued interest in BST among state agencies, river authorities, and stakeholder groups across Texas emphasizes the necessity of maintaining statewide BST analytical infrastructure. Advances in BST science and methodology remain an important component of the state BST analytical infrastructure and program. This includes needed maintenance and repairs of analytical equipment, and continued support, training, and retention of skilled personnel to facilitate using novel NGS techniques. With recent personnel changes at UTHealth H SPH and TWRI, there is also a near-term need for increased interaction among laboratories to facilitate the transition. To meet the needs of the state, BST analytical capabilities will be maintained at both UTHealth H SPH and AgriLife SCSC BST laboratories. Financial support will be used to maintain lab personnel at UTHealth H SPH and AgriLife SCSC, continue refinement and evaluation of the Texas *E. coli* BST library, continue work on marker development and evaluation, and support targeted NGS BST analysis. Utilizing NGS techniques to screen water bodies for bacterial pathogens also provides opportunities to better assess the influence of different fecal sources on the distribution of specific microbial pathogens in surface waters, therefore informing watershed management practices. While measuring water quality for FIB and BST informs of pollutant sources, directly evaluating a water body for an array of microbial pathogens provides the potential for rapidly identifying specific exposure risks.

There are two parallel aims to this project which include: 1) conduct BST analyses using NGS techniques for water samples gathered from nine sampling sites in the Medina River Above Medina Lake watershed and 2) conduct NGS analyses for water samples gathered at the nine sampling sites to provide an overview of potential pathogens present. Water samples will be collected over 12 months to provide an overview of different fecal sources impacting these water bodies. Additionally, four storm samples will be collected at two sampling sites during this period. Grab samples will be collected concurrently to measure for *E. coli*, which will be incorporated into the metagenomics analysis and evaluation of NGS techniques as a potential tool for the watershed management toolbox. AgriLife SCSC personnel will work with BCRAGD to 1) filter collected water samples to collect microbial biomass, 2) extract microbial DNA, and 3) conduct metagenomic sequencing using NGS technology. Generated data will be compared against sequence data from known-source samples also collected in this project to identify the sources of fecal microorganisms. In consultation with stakeholders, up to 10 potential sources of fecal contamination in the watershed will be identified. From each of these sources, 10 unique samples will be collected (up to 100 total known-source samples) and sequenced as described above to generate a known-source microbiome sequence library. Bioinformatics will then be used to compare NGS data from water samples against the known-source NGS data for source determination. Generated data will also be compared against publicly available genomic databases to identify the presence of pathogenic microorganisms. AgriLife SCSC will work with the Texas A&M Institute for Genome Sciences and Society (TIGSS) core facility or comparable laboratory for sequence analysis and bioinformatics training needed to interpret the metagenomics data for a water quality management context. Findings from the study will be evaluated for application to watershed management and how information can be translated to the stakeholder level.

The proposed project will represent continued use of NGS-based approaches for watershed source delineation in the Texas BST Program. This approach will be a valuable addition to the BST toolbox, complementing current library-independent tools. The second aim of using NGS techniques for pathogenic microorganism detection will complement source tracking efforts by attempting to directly identify pathogens of public health concern. Comparing findings from these two aims will improve the utilization and interpretation of NGS-based work for future water management. Further, it has the potential to provide information similar to that obtained using culture-based, library-dependent approaches, but at substantially lower cost due to rapid advances in sequencing technologies.

The project will also include continued development and refinement of the Texas *E. coli* BST Library, specifically to evaluate the delineation of feral hogs. Existing DNA fingerprints of feral hogs in the library will be evaluated to determine if a four-way split of source classes, including human, domestic animals, wildlife, and feral hogs is feasible.

BST results, from previous studies and this one, will be integrated into the QMRA framework to not only inform of human health risks associated with contact recreation, but also assist in informing watershed management practices. The QMRA will follow methods described in Haas et al. (2014). Estimated risk outputs will be evaluated and the feasibility of recommendations for incorporating QMRA into future watershed management across the state of Texas will be developed.

Furthermore, NGS data generated from this project will be deposited in the National Center for Biotechnology Information GenBank database and will be a valuable asset to other water quality projects. Discussing and sharing findings from this novel project are critical towards advancing watershed management science and water quality protection. TWRI, AgriLife SCSC, and UTHealth H SPH will develop materials concerning the project and the application of the science and distribute the information to water resource managers, natural resources agencies, universities and other stakeholders. TWRI will include information on the project in its publications. A final report will be developed that describes the findings of this study and its application for watershed management.

Table A6.1 Project Plan Milestones

Task	Project Milestones	Agency	Start *(Project Month#)*	End *(Project Month#)*
2.1	Develop QAPP	TWRI, UTHealth H SPH, SAML, BCRAGD	1	24
2.2	Submit revisions/amendments to QAPP	TWRI	1	24
3.1	Develop a targeted list of needed species for fecal sample collection and plan for their collection and delivery	TWRI, UTHealth H SPH, SAML, BCRAGD	2	5
3.2	Collect up to 100 fecal samples from the Medina River Above Medina Lake watershed	BCRAGD	5	24
3.3	Collect monthly grab samples from 9 selected monitoring sites in the Medina River Above Medina Lake watershed	BCRAGD	5	16
4.1	Maintain analytical and laboratory equipment to support BST analyses.	UTHealth H SPH, SAML	1	24
4.2	Retain lab personnel to maintain laboratory operating capacities and technical expertise to conduct BST studies state-wide.	UTHealth H SPH, SAML	1	24
4.3	Perform BST analyses for a combined total of approximately 116 samples from sites in the Medina River Above Medina Lake Watershed.	UTHealth H SPH, SAML	5	24
4.4	Determine water sample source contributions through bioinformatics evaluation of water sample NGS data against known-source NGS data.	UTHealth H SPH, SAML	5	24
4.5	Deposit NGS data in a publicly available database.	SAML	5	24
4.6	Evaluate differences in BST data and findings between the Medina River Above Medina Lake and other comparable watersheds in the state.	UTHealth H SPH, SAML	5	24
4.7	Integrate the BST results from the project into a QMRA to evaluate	UTHealth H SPH, SAML	5	24

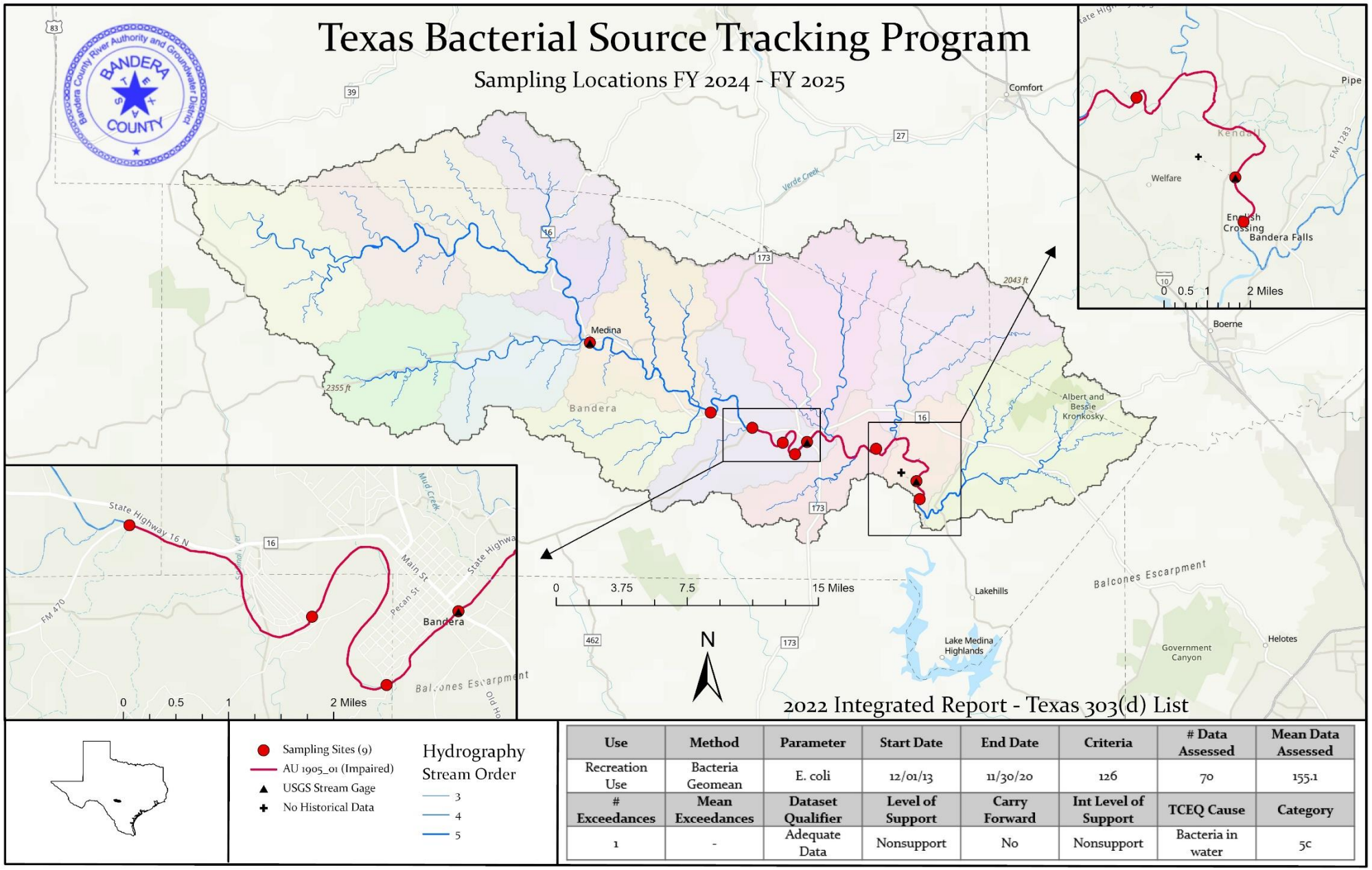
	the public health significance of the project's data.			
5.1	Perform metagenomics sequencing on DNA from water samples to ID detected organisms	SAML	5	24
5.2	Compare metagenomics, *E. coli*, water quality, and land use data to ID findings relative to watershed management	TWRI, SAML	5	24
5.3	Evaluate NGS findings for future use in watershed management	SAML	5	24
5.4	Consult with NGS experts to better understand its potential for advancing water quality management in Texas.	SAML, TWRI	5	24
5.5	Deposit NGS data in GenBank database	SAML	20	24
6.1	Evaluate and refine the statewide *E. coli* BST Library.	UTHealth H SPH, SAML	5	24
7.1	Host and maintain project website	TWRI	1	24
7.2	Promote use of and provide resources on BST	TWRI, UTHealth H SPH, SAML	1	24

Table A6.2 Water Quality Monitoring Stations

Routine Monitoring Sites							
Station ID	**Site Description**	**Latitude Longitude**	**Sample Matrix**	**Monitoring Frequencies (per year)**		**TCEQ Monitoring Station?**	**Associated Stream Gage?**
				Grab	**Sample Type**		
MP-3.01	Medina River at Moffett Park	29.79410671 -99.24870732	water	12	Routine	☒	☒
MR-3.06	Medina River at Peaceful Valley Rd.	29.74399081 -99.14880424	water	12	Routine	☐	☐
MR-3.04	Medina River at Tarpley Crossing	29.73318431 -99.11435803	water	12	Routine	☒	☐
MR-2.07	Medina River at Mayan Ranch	29.72215813 -99.0891492	water	12	Routine	☒	☐
MR-2.05	Medina River at 1st Street Bridge	29.71392485 -99.07909013	water	12	Routine	☐	☐
MR-2.03	Medina River at City Park	29.72269048 -99.06947441	water	12	Routine	☒	☒
MR-1.02	Medina River at Bridlegate Park	29.71676762 -99.01224312	water	12	Routine	☐	☐
MR-1.015	Medina River at USGS 08178980	29.694389 -98.979306	water	12	Routine	☐	☒
MR-1.01	Medina River at English Crossing	29.6817604 -98.97588559	water	12	Routine	☒	☐

Storm Based Sampling Sites							
Station ID	**Site Description**	**Latitude Longitude**	**Sample Matrix**	**Monitoring Frequencies (per year)**		**TCEQ Monitoring Station?**	**Associated Stream Gage?**
				Grab	**Sample Type**		
MR-2.03	Medina River at City Park	29.72269048 -99.06947441	water	4	Biased Flow	☒	☒
MR-1.01	Medina River at English Crossing	29.6817604 -98.97588559	water	4	Biased Flow	☒	☐

Figure A6.3 Map of Sampling Locations



Use	Method	Parameter	Start Date	End Date	Criteria	# Data Assessed	Mean Data Assessed
Recreation Use	Bacteria Geomean	E. coli	12/01/13	11/30/20	126	70	155.1
# Exceedances	**Mean Exceedances**	**Dataset Qualifier**	**Level of Support**	**Carry Forward**	**Int Level of Support**	**TCEQ Cause**	**Category**
1	-	Adequate Data	Nonsupport	No	Nonsupport	Bacteria in water	5c

Section A7: Quality Objectives and Criteria for Data Quality

The objective of this section is to ensure that data collected meets the DQOs of the project. The current project has a significant focus on conducting BST analytical procedures to support watershed planning in the Medina River Above Medina Lake watershed. Project objectives include the continued development, evaluation, and refinement of the Texas *E. coli* BST Library through investigation of the diversity of source specific, cosmopolitan, transient, and naturalized *E. coli* isolates as the library is expanded; continued development and evaluation of new source-specific bacterial markers for library independent BST; expansion of the Texas *E. coli* BST Library through the collection of additional known source samples of *E. coli;* and conducting BST analysis in support of watershed planning in the Medina River Above Medina Lake watershed. Sampling conducted in conjunction with this project is primarily associated with collection of known source fecal samples for assessment and use in expanding the Texas *E. coli* BST Library. Water sampling will be conducted consistent with the most recent version of the Texas Commission on Environmental Quality's (TCEQ) *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods* (TCEQ, 2012). Routine grab samples will be collected on a monthly basis. Only non-regulatory water grab samples will be collected under this project. This project will also integrate NGS and QMRA to evaluate the implications of combining these approaches and the utility of data generated for its relevance in watershed and water quality management activity.

Water samples collected will be preprocessed by BCRAGD and transported to SAML for bacteria isolation and BST analysis. Methods outlined in Tables A7-1 and B2-1 will be employed. Appropriate DQOs, performance criteria, and QA/QC requirements for this analysis are also reported in Tables A7-1 and B2-1.

After sample analysis, BST results will be utilized in a QMRA to develop human health risk estimates for a range of exposure scenarios (e.g. primary contact and secondary contact activities, etc.). The QMRA framework will follow methodology and procedures recommended by the United States Environmental Protection Agency (USEPA) (USEPA, 2010) and Haas et al. (2014). Parameters utilized in the risk assessment (e.g. ingestion volume while swimming, etc.) will be retrieved from published literature or experts in the field. Risk simulations will be developed utilizing the Excel® (Microsoft Corp., Redmond, WA) add-in software Crystal Ball Pro® (Oracle Corp., Redwood Shores, CA). Human health risk estimates will be compared to previously published QMRA studies to identify accuracy and validity of risk estimates.

Precision

Precision of laboratory data is a measure of the reproducibility of a result from repeated analyses. It is strictly defined as a measure of the closeness with which multiple analyses of a given sample agree with each other. For quantitative microbiological analyses, the method to be used for calculating precision is the one outlined in *Standard Methods for the Examination of Water and Wastewater,* 23rd Edition, section 9020 B.8.b (2018).

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

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

Representativeness

One subtask of this project is to expand the Texas *E. coli* BST Library so it is more representative of the *E. coli* isolates found in known source samples throughout Texas watersheds. The ability to reach this goal is tempered by the availability of time and funding. To maximize resources, only one fecal sample per animal will be collected. Samples will be collected from animals in different locations throughout the Medina River Above Medina Lake watershed and immediately adjacent areas as needed.

Accuracy

Accuracy is a statistical measurement of correctness and includes components of systemic error. A measurement is considered accurate when the result reported does not differ from the true situation. Performance limits for all measured parameters are specified in Table A7.1.

Comparability

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

Completeness

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

Table A7.1 Data Quality Objectives for Measurement Data

Parameter	Units	Method Type	Method	Method Description	Parameter Code	AWRL[1]	Precision of Laboratory Duplicates	Percent Complete[3]
E. coli, IDEXX	MPN/100 mL	Enzyme substrate coliform test	9223 B*	*E. coli* enumeration	31699	1	0.5**	90
E. coli IDEXX, Holding Time	hours	NA	NA	NA	31704	NA	NA	90
DNA sequencing	Relative abundance	DNA sequence	MinION, MiSeq	DNA sequencing	NA	NA	NA	90

[1] minimum detection limits for field parameters represent manufacturer specifications and will be used for the AWRL in this instance.
[2] Manufacturer specifications are presented for accuracy limits and method detection limits for field parameters.
[3] The objective is for 90% of the data to be collected.
*American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), Standard Methods for the Examination of Water and Wastewater, 23rd Edition, 2018.
** This value is not expressed as a relative percent difference. It represents the maximum allowable difference between the logarithm of the result of a sample and the logarithm of the duplicate result. See Section B5.

Section A8: Special Training Requirements/Certifications

All personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. Personnel involved in this project have been trained in the appropriate use of field equipment, laboratory equipment, laboratory safety, cryogenics safety, and all applicable SOPs. One of the objectives of this project is the continued support, training, and retention of skilled personnel. To meet the needs of the State, BST analytical capabilities will be maintained at both UTHealth H SPH and SAML BST laboratories. Training needs for each individual laboratory's personnel will be coordinated to ensure appropriate technology transfer and comparability of BST data.

Section A9: Documentation and Records

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 as outlined in Table A9.1. In addition, UTHealth H SPH and SAML will archive electronic forms of all project data for at least five years. All electronic data are backed up on an external hard drive monthly, compact disks weekly, and is simultaneously saved in an external network folder and the computer's hard drive. A blank CAR form is presented in Appendix A and a blank COC record and Sheets of Lading for Fecal Specimen Transport are presented in Appendix B.

Quarterly Progress Reports (QPRs) will note items or areas identified as potential problems and any variations or supplements to the QAPP. CARs will be utilized when necessary. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP. All QPRs and QAPP revisions will be distributed to personnel listed in Section A3.

Table A9.1 Project Documents and Records

Document/Record	Location	Retention	Form
QAPP, amendments, and appendices	TWRI/SAML/ UTHealth H SPH /BCRAGD	5 years	Electronic
Chain of custody records	SAML/ UTHealth H SPH	2 years	Paper/Electronic
Sheets of Lading for Fecal Specimens	SAML/ UTHealth H SPH	2 years	Paper/Electronic
Corrective action reports	TWRI	2 years	Electronic
Field notes	BCRAGD	2 years	Paper/Electronic
Bacteriological data sheet	SAML/ UTHealth H SPH	2 years	Paper/Electronic
Laboratory QA manuals and/or SOPs	SAML/ UTHealth H SPH	5 years	Paper/Electronic
Lab equipment calibration records & maintenance logs	SAML/ UTHealth H SPH	2 years	Paper/Electronic
Lab data reports/results	SAML/ UTHealth H SPH	5 years	Paper/Electronic
Quarterly progress reports/final report/data	TWRI	5 years	Paper/Electronic

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

QAPP Revision and Amendments

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

QAPP amendments 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; Written requests for amendments are directed from the TWRI Project Leader or designee to the TSSWCB PM and are effective immediately upon approval by the TSSWCB PM and QAO. Amendments to the QAPP and the reasons for the changes will be documented and distributed to all individuals on the QAPP distribution list by the TWRI Project Leader or designee. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

Section B1: Sampling Process Design (Experimental Design)

The sampling to be conducted in conjunction with this project is associated with supporting the expansion of the Texas *E. coli* BST Library through the collection of known sources of fecal matter from the Medina River Above Medina Lake watershed. The primary sampling design approach is to collect 100 fecal samples from the watershed comprised of targeted species that will be determined by SAML, UTHealth H SPH, BCRAGD, and TWRI, cooperatively. If a source category is underrepresented in that project's data set, additional fecal samples may be collected by BCRAGD to fill gaps in the available known sources of fecal material. Sampling will focus on specific species or classes (e.g. avian wildlife) and will be conducted where possible. No specific geographic locations within the watershed will be specified for these samples to be collected from.

Water samples from the Medina River Above Medina Lake watershed processed under this project will be collected as grab samples and delivered to the SAML for DNA extraction and NGS sequencing. A total of 108 samples will be collected on a monthly basis for a 1-year period, 12 at each of the 9 sites in the Medina River Above Medina Lake watershed. Additionally, 4 storm samples will be collected at 2 sampling sites during this period for a total of 8additional samples. Grab samples will be preprocessed and stored by BCRAGD until delivered and further processed by SAML. The samples will then be divided between SAML and UTHealth H SPH for further isolate and BST analysis.

Section B2: Sampling Method Requirements

Fecal Sample Collection

All collection and handling of fecal specimens conducted by BCRAGD will be performed using all safety precautions (i.e.: wearing protective gear) and will be strictly enforced. Specimens will be handled aseptically to ensure sample quality and minimize exposure of personnel to pathogens. All fecal material and waste collected will be placed in screw capped sterile containers (Table B2.1) or sterile Whirl-Pak bags. Containers will be labeled with: Name of collector, date, and species before collection. Fecal specimens will be placed in an insulated cooler and transported to the BCRAGD Lab for pre-processing. 30 grams of fecal material is the goal weight of feces to be collected from each animal. Should 30 grams not be achievable due to animal size or other factors, as much fecal matter as possible will be collected. At least 0.1 grams of feces must be collected to be useful for DNA extraction.

Fecal sample collections are described in Appendix C-1. To ensure fresh samples of known origin, fecal samples will be obtained using one of three methods: a) collected from animals visually observed defecating by technician; b) collected from cages of trapped animals; c) collected from intestines of animals. All fecal samples will be stored at -20C until delivery to SAML for archival and long-term storage at -80C.

Water Sample Collection

BCRAGD will follow the most recent versions of the field sampling procedures documented in the TCEQ (2012). Water samples will be collected directly from the stream (midway in the stream channel) in most cases. Water samples used BST and NGS 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. All samples will be transported in a container with ice to the laboratory for analysis.

Documentation of Field Sampling Activities

Recording Data

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

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

Each fecal sample will be collected aseptically in a new, sterile fecal tube (Sarstedt, cat# 80.734.311) or a new, sterile Whirl-Pak® bag as appropriate. Specimen sample containers will be labeled with:

a. Sampling date
b. Sampling time
c. Animal species
d. Sample location (e.g., global position system coordinates [preferred] or town, city, and/or county)
e. Sample collector's name, initials
f. Any other pertinent information, e.g., sex of animal; juvenile or adult

All the sample information will be logged into a field log. Samples should be refrigerated (~4°C) or kept on ice following collection and shipped to the designated laboratory on ice within 24 hours of collection. If shipping within 24 hours isn't feasible, samples should be frozen (-20C) until delivery on dry ice to the lab. See SOP in Appendix C for complete protocol.

Table B2.1 Sample Volume, Container Types, Minimum Sample Volume, Preservation Requirements, and Holding Time Requirements.

Parameter	Matrix	Container	Preservation	Temperature	Sample Volume	Holding Time
Fecal Specimen	Feces	Sterile fecal sample tube	None	4°C	30g[1]	24 hours
E. coli	Water	Sterile Whirl-Pak Bag	Ice	≤4°C	400 mL	24 hours

[1] 30 grams is the goal weight for fecal matter collection; however, should it not be possible to collect 30 grams of feces, as much material as possible will be collected. 0.1 grams is the minimum allowable weight of fecal material to be collected.

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

Section B3: Sample Handling and Custody Requirements

Chain-of-Custody

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

Sample Labeling

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

Sample Handling

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

Failures in Chain-of-Custody and Corrective Action

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

Section B4: Analytical Method Requirements

NGS Analysis

For NGS analysis, fecal samples will be frozen at -20C as soon as possible (within no more than 24 hr after collection) and archived at -80C upon delivery to SAML. For water samples, samples will be filtered upon receipt in lab and the filtered biomass immediately frozen and stored at -80C. DNA will be extracted using commercial kits following the manufacturer's protocols. Extracted DNA will be sequenced using the MinION system and TIGSS core facility following manufacturer protocols.

BST Analysis

The analytical methods utilized in BST analysis and sample preparation are listed in Table B4.1 and Table A7.1 and described in detail in Appendix C. All laboratory sampling areas and equipment will be sterilized with at least one or in any combination of the following methods: ethyl alcohol, bleach, ultraviolet (UV) light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal.

Water samples collected and pre-processed by BCRAGD (Appendix C-2) will be delivered on dry ice to SAML for archival and further processing. BCRAGD will filter and preserve three separate 100 mL aliquots for each sample.

QMRA Methods Development

UTHealth H SPH, SAML and TWRI will utilize QMRA methods described in the published literature to develop an approach integrating BST results and QMRA to provide a human health context for analyzed BST data. The best available and scientifically defensible methods will be used to assess the human health risks associated with recreation in waters impacted by different fecal sources, as described by the BST results. The QMRA approach will follow previously described guidelines (USEPA, 2010; Haas et al., 2014) to develop an integrated BST-QMRA framework to evaluate the human health risks associated with recreational activities at the Medina River Above Medina Lake sites. The framework will include four components: hazard characterization, exposure assessment, dose-response model(s), and risk characterization. Further, this framework will be refined to be used at other sites in Texas to help inform water management decisions regarding pollution abatement, beach closures, public health risks, etc. The application of QMRA and BST data is another tool that once developed, will be a beneficial and cost-effective addition to the Texas BST toolbox to aid in watershed management decision-making.

Water Sampling and Analysis

The analytical methods are listed in Table A7.1 of Section A7. Laboratories collecting data under this QAPP are compliant with the TNI Standards and must be accredited in accordance with NELAP requirements for the matrix, method, parameter combinations listed in Table A7.1 of the QAPP on the date the samples are processed for analysis. In this project, these methods include the 9223 B enzyme substrate test for *E. coli* enumeration in water and fecal waste, as well as the BST methodology for library-dependent and library-independent isolate analysis.

SAML will analyze samples under this QAPP using the methods listed in Table A7.1 in non-potable water. Copies of laboratory Quality Assurance Manuals and SOPs are retained by the laboratory and are available for review. Laboratory SOPs are consistent with USEPA requirements as specified in the method.

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 UTHealth H SPH lead, 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.

Table B4.1 Laboratory Analytical Methods

Laboratory Parameter	Method	Equipment Used
E. coli in water	9223 B	Quanti-Tray Sealer PLUS, incubator
DNA extraction	Manufacturer Protocols	Vortex, centrifuge
DNA sequencing	Manufacturer protocols	MinION sequencer, TAMU TIGSS core facility

SOP = Standard Operating Procedure

Section B5: Quality Control Requirements

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

Method Specific QC requirements

QC samples, other than those specified later this section, are run (e.g., sample duplicates, surrogates, internal standards, continuing calibration samples, interference check samples, positive control, negative control, and media blank) as specified in the methods. The requirements for these samples, their acceptance criteria or instructions for establishing criteria, and corrective actions are method-specific.

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

Method blank

A method blank is a sample of matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. The method blanks are performed at a rate of once per preparation batch. The method blank is used to document contamination from the analytical process. For each of the analytical methods used in this project, method blanks should test negative for the target analytes/markers. In addition, no template negative controls will be analyzed for each batch of PCR. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the samples (e.g. reprocessing or data qualifying codes). In all cases the corrective action must be documented.

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

Failures in Quality Control and Corrective Action

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

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

To minimize downtime of all measurement systems, spare parts for laboratory equipment (Table B6.1) will be kept in the laboratory (when feasible), and all laboratory equipment will be maintained in working condition. All laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in Standard Methods for the Examination of Water and Wastewater, 22nd Edition. Maintenance and inspection logs will be kept on each piece of laboratory equipment. Records of all tests, inspections, and maintenance will be maintained and log sheets kept showing time, date, and analyst signature. These records will be available for inspection by the TSSWCB. Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. CARs will be maintained by the TWRI Project Lead.

Table B6.1 Equipment Inspection and Maintenance Requirements

Equipment	Relevant Testing, Inspection & Maintenance Requirements
Thermometers	SM 9020 B 3.a
Water deionization units	SM 9020 B 3.d
Media dispensing apparatus	SM 9020 B 3.f
Autoclaves	SM 9020 B 3.h
Refrigerator	SM 9020 B 3.i
Ultra Low Freezer	SM 9020 B 3.j
Membrane filter equipment	SM 9020 B 3.k
Ultraviolet sterilization lamps	SM 9020 B 3.l
Biological safety cabinet	SM 9020 B 3.m
Incubators	SM 9020 B 3.o
Glassware and plastic ware	SM 9020 B 3.a
Utensils and containers	SM 9020 B 3.b
Dilution water bottles	SM 9020 B 3.c

Section B7: Instrument Calibration and Frequency

Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. The instruments requiring calibration are listed below in Table B7.1. All calibration procedures will meet the requirements specified in the USEPA-approved methods of analysis. The frequency of calibration as well as specific instructions applicable to the analytical methods recommended by the equipment manufacturer will be followed. All information concerning calibration will be recorded in a calibration logbook by the person performing the calibration and will be accessible for verification during either a laboratory or field audit.

All instruments or devices used in obtaining environmental data will be used according to appropriate laboratory or field practices. Written copies of SOPs are available for review upon request.

Standards used for instrument or method calibrations shall be of known purity and be National Institute of Standards and Technology (NIST) traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society or reagent grade quality, or of the best attainable grade. All certified standards shall be maintained and traceable with certificates on file in the laboratory. Dilutions from all standards will be recorded in the standards logbook 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 logbook.

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

Table B7.1 Instrument Calibration Requirements

Equipment	Relevant Calibration Requirement
MinION Sequencer	Per manufacturer

Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

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

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

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

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

Section B10: Data Management

Laboratory Data

All field samples (known-source fecal samples) will be logged upon receipt, COC forms (if applicable) will be checked for number of samples, proper and exact identification number, signatures, dates, and type of analysis specified. TSSWCB will be notified if any discrepancy is found and laboratory analysis will not occur until proper corrections are made. All samples will be stored at 4ºC until analysis. Bacteriological samples will be given a unique identification number and logged into a database used to store field data. All backup and safety features of this database are the same as explained above. Enumerated bacteriological data will be manually entered into the database system for electronic storage. Per lab SOPs, at least 10% of all data manually entered in the database will be reviewed for accuracy by the Project Lead to ensure that there are no transcription errors. Hard copies of data will be printed and housed at the generating laboratory for a period of five years. Any COC's and bacteriological records related to QA/QC of bacteriological procedures will be housed at UTHealth H SPH and SAML.

DNA Sequence Data

DNA sequence data, and corresponding sample metadata, will be deposited in the US National Library of Medicine, National Center for Biotechnology Information, GenBank® database and made publicly available. Accession number for the sequence data will be included in all related reports and publications.

Sample Delivery to Other Laboratories

Fecal samples for BST analysis will be collected and logged using the procedures described above in the field collection and lab data sections. The Technician ensures that these samples are handled according to procedures laid out in this QAPP and that COC forms are correctly filled out for sample delivery to the UTHealth H SPH and SAML as appropriate. The Technician hand-delivers, or ships the samples, the appropriate Sheets of Lading for Fecal Specimen Transport (Appendix B) and COC forms to the UTHealth H SPH and SAML labs via FedEx in an appropriately labeled container that maintains appropriate sample temperatures with the use of blue ice. Once the samples are received at the lab, the COC forms are updated, and the Technician is notified of the samples receipt.

Data Validation

Following review of laboratory data, any data that is not representative of environmental conditions, because it was generated through poor field or laboratory practices, will not be submitted to the TSSWCB. This determination will be made by the UTHealth H SPH Project Co-Lead or SAML AgriLife SCSC Project Co-Lead, BCRAGD QAO, TWRI QAO, TSSWCB QAO, and other personnel having direct experience with the data collection effort. This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the checks specified in Table D2.1.

Data Reporting

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

Data Dissemination

At the project's conclusion, the TWRI Project Lead will provide a copy of the complete project electronic database to the TSSWCB PM, along with the final report. TSSWCB may elect to take possession of all project records or records will be maintained according to the Project Records retention schedule in Table A.9. Summaries of the data will be presented in the final project report. TSSWCB may disseminate validated data and reports.

Section C1: Assessments and Response Actions

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, UTHealth H SPH	Pass/Fail equipment testing	Repair or replace
Data completeness	As needed	SAML, UTHealth H SPH	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

Corrective Action

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

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

Section C2: Reports to Management

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

TWRI will work with AgriLife SCSC, BCRAGD, and UTHealth H SPH to develop a Final Report for submission to the TSSWCB that summarizes activities completed, conclusions reached during the project, and the extent to which project goals and measures of success have been achieved.

Section D1: Data Review, Validation, and Verification

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

The procedures for verification and validation of data are described in Section D2, below. 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 and Verification Methods

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

Verification, validation, and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified (listed by task in Table D2.1) are evaluated against project specifications (Section A7 and Section B5) and are checked to ensure the verification of raw data for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data or identified using computer-based statistical software. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues that can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TSSWCB QAO to establish the appropriate course of action, or the data associated with the issue are rejected. Performance of these tasks is documented by completion of the data review checklist (Appendix D).

Project Leaders and TWRI are responsible for validating that the verified data are scientifically sound, defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to the TSSWCB.

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
Sample documentation complete	X	X	X
Field QC results within acceptance limits			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
QC samples analyzed at required frequencies		X	X
QC samples within acceptance limits		X	X
Instrument readings/printouts		X	X
Calculations		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
Absence of transcription error	X	X	X
Reasonableness of data	X	X	X
Electronic submittal errors		X	X
Sampling and analytical data gaps	X	X	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

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. Corrective Action Reports will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the data quality objectives of the project and the informational needs of water quality agency decision-makers and watershed stakeholders.

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only BST data that has met the data quality objectives described in this QAPP will be reported and included in the final project report. Since BST is an evolving science and no USEPA-approved protocols currently exist, a discussion of the uncertainties surrounding source identification and the appropriate use of BST results will be included in the project final report. Data and information produced through this project will provide needed information pertaining to Texas BST efforts.

References

Byappanahalli, M. N., Shively, D. A., Nevers, M. B., Sadowsky, M. J., Whitman, R. L. 2003. Growth and Survival of *Escherichia coli* and enterococci populations in the macro-alga *Cladophora* (Chlorophyta), *FEMS Microbiology Ecology*, 46(2), 203-211. https://doi.org/10.1016/S0168-6496(03)00214-9

Hass, C. N., Rose, J. B., Gerba, C. P. 2014. Quantitative Microbial Risk Assessment. 2nd Edition. John Wiley & Sons. Hoboken, NJ, USA.

Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation. 2018. Standard Methods for the Examination of Water and Wastewater: 9020 Quality Assurance/Quality Control. 23rd Editions. APHA Press. Washington, D.C., USA.

TCEQ. 2012. Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods. RG-415. Revised August 2012. https://www.tceq.texas.gov/publications/rg/rg-415

USEPA. 2010. Quantitative microbial Risk Assessment to Estimate Illness in Freshwater impacted by Agricultural Animal Sources of Fecal Contamination. Office of Water, United States Environmental Protection Agency. Washington, D.C., USA.

USEPA. 2014. Method 1603: *Escherichia coli (E. col*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Augar (Modified mTEC). Office of Water, United States Environmental Protection Agency. Washington, D.C., USA.

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APPENDIX A: Corrective Action Report

Corrective Action Report
CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

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

Possible causes:
_____
_____
_____

Recommended corrective action:
_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:
_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Project Leader:_____

Quality Assurance Officer:_____

APPENDIX B: Chain of Custody Record & Sheets of Lading for Fecal Specimen Transport Template

CHAIN OF CUSTODY RECORD

Project:	**Remarks:**
Name and signature of collector:	**Air bill #**

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

Relinquished by:	Date:	Time:	Received by:	Date:	Time:
Laboratory Notes:					
Media Code: (**FS**) Fecal Sample; (**SS**) Sewage Sample					

Sheets of Lading for Fecal Specimen Transport

(Collector's Organization)

Texas BST Program (FY24-FY25)

(Collector's Name and title)
(Collector's Phone Number)

In case of EMERGENCY:
(Contact name and number)

Date:_____Time:_____

Sample: Fecal **Hazard:** Bacteria

Species/ Animal:_____

Photo: Yes No

GPS (or other location note): Lat_____Long_____

_____

Other Info:_____

Technician:_____

APPENDIX C: BST Standard Operating Procedures

C-1: Collection of Fecal Samples for Bacterial Source Tracking

April 15, 2024

Elizabeth Casarez
University of Texas Health Science Center Houston (UTHealth) School of Public Health El Paso

1.0. PURPOSE AND APPLICABILITY

The purpose of this Standard Operating Procedure (SOP) is to establish a uniform procedure for the collection and transport of fecal samples to the laboratory for subsequent isolation of *E. coli* for Bacterial Source Tracking (BST) analyses.

2.0. SUMMARY OF THE METHOD

Fresh fecal, sewage, or septage samples are collected, immediately placed at 4°C, stored at -20°C to -80°C (for microbiome-based BST), and shipped/transported to the appropriate BST laboratory as soon as possible.

3.0. HEALTH AND SAFETY WARNINGS

Fecal, sewage, or septage samples may contain pathogenic microorganisms. The sampler should treat all such samples as though each contained a chemical and/or a biological agent that could cause illness. The sampler should wear protective gloves and handle containers with care. The sampler should exercise special caution to avoid environmental hazards such as animals (e.g., snakes), extreme climatic conditions, and automobiles (if collecting a sample near a major road).

4.0. INTERFERENCES

Possible issues include the collection of old, unidentifiable, or contaminated samples. Only fresh fecal samples of known origin should be collected. Samples should be carefully collected to avoid contamination from the surrounding environment (soil, etc.). Specific suggestions for avoiding these interferences are provided in the procedures section of this SOP.

5.0. PERSONNEL QUALIFICATIONS

This SOP is written for persons with a thorough knowledge of field sampling procedures and a basic understanding of microbiological procedures, especially aseptic technique.

6.0. EQUIPMENT AND SUPPLIES

6.1 Sterile fecal tubes (Sarstedt, cat# 80.734.311) or similar containers
6.2 Sterile spatulas, or similar, for collection of samples
6.3 Sterile plastic loops (optional)
6.4 Sterile scalpels (optional)
6.5 Sterile bottles (optional; for wastewater collection)

6.6 Whirl-Pak bags, or similar
6.7 Cooler with ice, blue ice, and/or dry ice for transport of samples
6.8 Refrigerator (~4°C) and/or freezer (-20°C to -80°C). If using -20°C freezer, make sure it is manual defrost.

7.0. PROCEDURAL STEPS

7.1. Only fresh fecal samples of known origin should be collected. Specifically, fecal samples should be obtained in one of five ways:
a. Collected from animals visually observed defecating.
b. Collected from trapped animals.
c. Collected from intestines of animals legally harvested.
d. Collected from the intestines of animals recently killed by cars (within 24 hours).
e. Human (wastewater) samples collected from individual septic tanks, composite septic samples from pump trucks, from wastewater treatment plant influent (for plants with secondary disinfection or lagoon treatment), or from lagoon treatment effluents.

7.2. Samples should be carefully collected to avoid contamination. Samples on the ground should be collected with a sterile spatula, or similar device, while avoiding collection of material in contact with soil or other possible sources of contamination. Intestinal samples should be collected from animals by using sterile loops inserted anally or by cutting into the intestine using a sterile scalpel. Wastewater samples can initially be collected with sterile bottles or other suitable device and then transferred to the fecal tubes described below.

7.3. Each fecal sample should be placed in a new, sterile fecal tube, or similar container. Tubes should be filled approximately ¾ full (can provide less material for smaller animals).

7.4. Samples should be placed in a cooler on ice and/or refrigerated (~4°C) following collection.

7.5. At the time of sampling, record detailed information on the tube regarding the sample including:
a. Sampling date
b. Sampling time
c. Animal species
d. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
e. Sample collector’s name/initials
f. Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle

7.6. Notify the appropriate lab via email or phone as soon as possible (prior to or immediately following sample collection) with an estimated number of samples that will be shipped and the expected date of shipment. This will allow the lab to make appropriate preparations to process the samples immediately upon arrival. BST Laboratory contact information is below:

UTHealth
Carlos Monserrat; carlos.monserrat@uth.tmc.edu; 915-304-9122

SAML
Terry Gentry; tjgentry@tamu.edu; 979-321-5918

7.7. For *E. coli* culture-based BST, samples should be shipped (at 4ºC) as soon as possible (within 3 days) to the appropriate lab (addresses below). Ship samples (and COCs) in insulated coolers (marked on outside to indicate that contents are perishable) with sufficient ice packs to maintain ~4°C. 'Blue-ice' or freezer blocks should be used to keep the samples cool, but not frozen during transport. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.

For Microbiome-based BST, samples should be transported to the lab at 4ºC within 24 hours. As soon as possible, samples should be stored at -20ºC to -80 ºC. Ship samples (and COCs) in insulated coolers (marked on outside to indicate that contents are perishable) with sufficient dry ice to maintain frozen conditions. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.

Shipping addresses for BST Laboratories are:

UTHealth
Carlos Monserrat
UT-Houston School of Public Health
800 Canal Road
El Paso, TX 79901
915-304-9122

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

979-845-5604

7.8. Notification of shipment should be sent to the appropriate lab via email or phone (see contact info above) no later than the day of overnight shipping. Notification should include tracking number and contact person for confirmation upon receipt of samples.

8.0. QUALITY ASSURANCE AND QUALITY CONTROL

Care should be exercised to avoid the interferences listed in section 4.0. Any potential issues for the BST Laboratory to consider should be noted on the COC form. Following collection, samples should be maintained at ~4°C (for E. coli isolation) or frozen (for microbiome-based BST) and transported/shipped to the BST Laboratory as soon as possible in order to minimize changes in microbial composition of the samples.

9.0. REFERENCES

Casarez, E. A., S. D. Pillai, J. B. Mott, M. Vargas, K. E. Dean and G. D. Di Giovanni. 2007. Direct comparison of four bacterial source tracking methods and use of composite data sets. J. Appl. Microbiol. 103:350-364.

Di Giovanni, G. D., E. A. Casarez, T. J. Gentry, E. C. Martin, L. Gregory, and K. Wagner. 2013. Support analytical infrastructure and further development of a statewide bacterial source tracking library. TR-448. Texas Water Resources Institute, College Station, TX.

10.0. REVISION HISTORY

Revision	Date	Responsible Person	Description of Change
1	June 2015	Elizabeth Casarez	Initial Release
2	August 2018	Lucas Gregory	Updated UTH Lab name and laboratory personnel
3	March 2019	Anna Gitter/Elizabeth Casarez	Updated UTH laboratory personnel
4	June 2020	Anna Gitter	Updated UTH laboratory personnel
5	August 2022	Anna Gitter	Updated UTH laboratory address
6	April 2024	Terry Gentry	Modified E. coli isolation SOP for use with Microbiome-based BST

C-2: Preprocessing of water samples for Microbiome- and NGS-Based BST

April 15, 2024

Joy Truesdale
University of Texas Health Science Center – Houston (UTHealth) School of Public Health El Paso

PURPOSE AND APPLICABILITY

The purpose of this Standard Operating Procedure (SOP) is to establish a uniform procedure for the initial processing of water samples for archival at -20°C to -80°C in preparation for future Bacterial Source Tracking analyses using DNA sequencing approaches.

1.0. SUMMARY OF THE METHOD

Water samples are passed through 0.2 µm-pore size membrane filters to collect microbial biomass. Filters, with attached biomass, are then and frozen at -20°C to -80°C until future analysis.

2.0. HEALTH AND SAFETY WARNINGS

Environmental water samples may contain pathogenic microorganisms. The analyst should treat all sources of wastewater as though each contained a chemical and/or a biological agent that could cause illness. The analyst should wear protective gloves and handle containers with care.

3.0. INTERFERENCES

Turbid waters may clog membrane filters before the desired volume of sample can be processed. If this occurs, filter as much water as possible (up to the desired volume) and record the amount of water filtered on bag/tube that the filter is placed into and on the chain-of-custody form.

4.0. PERSONNEL QUALIFICATIONS

This SOP is written for persons with a basic knowledge of laboratory and microbiological procedures.

5.0. EQUIPMENT AND SUPPLIES

5.1 Pipets (sterile), T.D. bacteriological, plastic, of appropriate volume
5.2 Sterile membrane filtration units (filter base and funnel), glass, plastic or stainless steel, wrapped with aluminum foil or kraft paper
5.3 Line vacuum, electric vacuum pump, or aspirator for use as a vacuum source (In an emergency or in the field, a hand pump or a syringe equipped with a check valve to prevent the return flow of air, can be used)
5.4 Filter flask, vacuum, usually 1 L, with appropriate tubing

5.5 Filter manifold to hold several filter bases (optional)
5.6 Flask for safety trap/filter placed between the filter flask and the vacuum source
5.7 Forceps, straight or curved, with smooth tips to handle filters without damage
5.8 Ethanol, methanol or isopropanol in a small, wide-mouth container, and cigarette lighter for flame-sterilizing forceps
5.9 Burner, Bunsen or Fisher type, or electric incinerator unit for sterilizing loops
5.10 Supor membrane filters, 0.2 µm pore size, sterile, white, 47 mm diameter (VWR cat # 28147-979)
5.11 Sterile, petri dishes,15 ml polypropylene centrifuge tubes, Whirl-Pak® bags, or equivalent
5.12 Freezer (-20°C or -80°C). If using -20°C freezer, make sure freezer is manual defrost.

6.0. PROCEDURAL STEPS

6.1 Within six hours of sample collection, water samples (100 ml) are filtered through 0.2 µm pore size Supor-200 filters
6.2 Discard filtrate and place the filter into a pre-labeled sterile petri dishes (or bag) using ethanol-flamed forceps and aseptic technique. If 100 ml of water cannot be filtered, record the volume filtered on the petri dishes and chain of custody form
6.3 Three separate 100 ml aliquots should be filtered for each sample. Use a new filter for each 100 ml aliquot and store the three filters separately.

6.3 Store samples at -20°C to -80°C. If using -20°C freezer, make sure the freezer is manual defrost.

7.0. QUALITY ASSURANCE AND QUALITY CONTROL

A method blank (sterile water or phosphate-buffered saline (PBS)) is processed with each batch of samples.

8.0. REFERENCES

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

9.0. REVISION HISTORY

Revision	Date	Responsible Person	Description of Change
1	June 2015	Joy Truesdale	Initial Release
2	March 2018	Maitreyee Mukherjee	Removing the use of guanidine isothiocyanate (GITC) as per the new DNA extraction method (SOP TXBST-07-revision 2)
3	August 2018	Lucas Gregory	Update UTH Lab name

4	August 2022	Anna Gitter	Updated UTH Lab name
5	April 2024	Terry Gentry	Modified Bacteroidales SOP for Microbiome and NGS analysis

APPENDIX D: Data Review Checklist & Data Summary Sheet

Data Review Checklist

Title of associated QAPP: _____

√, X, or N/A

Data Format and Structure

A. Are there any duplicate *Tag ID* numbers? _____
B. Are the *Tag prefixes* correct? _____
C. Are all *Tag ID* numbers 7 characters? _____
D. Are TCEQ station location (SLOC) numbers assigned? _____
E. Are sampling *Dates* in the correct format, MM/DD/YYYY? _____
F. Is the sampling *Time* based on the 24-hour clock (e.g. 13:04)? _____
G. Is the *Comment* field filled in where appropriate (e.g. unusual occurrence, sampling problems, unrepresentative of ambient water quality) and any punctuation deleted?

_____

H. *Source Code 1, 2* and *Program Code* are valid and used correctly? _____
I. Is the sampling date in the *Results* file the same as the one in the *Events* file? _____
J. Values represented by a valid parameter (*STORET*) code with the correct units and leading zeros? _____
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 numbers in the *Results* file that are not in the *Events* file? _____
N. Have confirmed outliers been identified? (with a ■1" in the Verify_flg field) _____
O. Have grab data (bacteria, for example) taken during 24-hr events been reported separately as RT samples? _____
P. Is the file in the correct format (ASCII pipe-delimited text)? _____

Data Quality Review

A. Are all the values reported at or below the AWRL? _____
B. Have the outliers been verified? _____
C. Checks on correctness of analysis or data reasonableness performed?
e.g.: Is ortho-phosphorus less than total phosphorus?
Are dissolved metal concentrations less than or equal to total metals? _____
D. 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

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 file 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 laboratory measurement systems that were not resolvable and resulted in unreportable data? If yes, explain on next page. _____

√ = Yes **X = No** **N/A** = Not applicable

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

_____

_____

_____

_____

_____

Date Submitted to TCEQ: _____
Tag ID Series: _____
Date Range: _____
Data Source: _____
Comments (attach README.TXT file if applicable):

_____

_____

_____

_____

Planning Agency's Data Manager Signature: _____

Date: _____