Appendix G to the Lower Neches River Basin/Neches Trinity Coastal Basin Clean Rivers Program FY 2024/2025 Quality Assurance Project Plan

Bacterial Source Tracking on Pine Island Bayou

Prepared by the Lower Neches Valley Authority (LNVA) in cooperation with the Texas Commission on Environmental Quality (TCEQ)

Effective: Immediately upon approval by all parties

Questions concerning this QAPP should be directed to:

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SS-A1 Approval Page

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Electronically Approved	11/12/2024
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Texas A&M University (TAMU) Soil and Aquatic Microbiology Laboratory

Electronically Approved 11/14/2024

Terry Gentry Date
TAMU Soil and Aquatic Microbiology Laboratory
Laboratory Manager

The LNVA will secure written documentation from each sub-tier project participant (e.g., subcontractors, other units of government) stating the organization's awareness of and commitment to requirements contained in this quality assurance project plan and any amendments or added appendices of this plan. Alternatively, additional signature blocks for sub-tier participants may be added to section A1. Signatures in section A1 will eliminate the need to adherence letters to be maintained. The LNVA will maintain this documentation as part of the project's quality assurance records and will ensure the documentation is available for review.

SS-A2 Table of Contents

List of Acronyms

AWRL	Ambient Water Reporting Limit
BST	Bacterial Source Tracking
CAP	Corrective Action Plan
CE	Collecting Entity
COC	Chain of Custody
CRP	Clean Rivers Program
DMRG	Surface Water Quality Monitoring Data Management Reference Guide
DM&A	Data Management and Analysis
DNA	Deoxyribonucleic Acid
DQO	Data Quality Objective
ELS	Environmental Laboratory Services
EPA	United States Environmental Protection Agency
ERIC	Enterobacterial Repetitive Intergenic Consensus
FY	Fiscal Year
LNVA	Lower Neches Valley Authority
LCS	Laboratory Control Sample
LCSD	Laboratory Control Sample Duplicate
LOQ	Limit of Quantitation
mTEC	Modified Membrane Thermotolerant *E. coli* Medium
PCR	Polymerase Chain Reaction
PIB	Pine Island Bayou
QA	Quality Assurance
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
QC	Quality Control
RP	RiboPrinting
SAML	Soil and Aquatic Microbiology Laboratory
SE	Submitting Entity
SOP	Standard Operating Procedure
SWQMIS	Surface Water Quality Monitoring Information System
TAMU	Texas A&M University
TCEQ	Texas Commission on Environmental Quality
TMDL	Total Maximum Daily Load
TSWQS	Texas Surface Water Quality Standards
UT	University of Texas

SS-A3 Distribution List

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SS-A4 Project/Task Organization

TCEQ

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Team Leader, Water Quality Standards and Clean Rivers Program
As described in the LNVA FY24–25 CRP QAPP, Section A4.

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As described in the LNVA FY24–25 CRP QAPP, Section A4.

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CRP Data Manager, DM&A Team
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Lawrence Grant Bassett
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Lower Neches Valley Authority

Jeannie Mahan
LNVA Project Manager & Acting Quality Assurance Officer
As described for the LNVA Project Manager and LNVA Quality Assurance Officer in the LNVA FY24–25 CRP QAPP, Section A4.

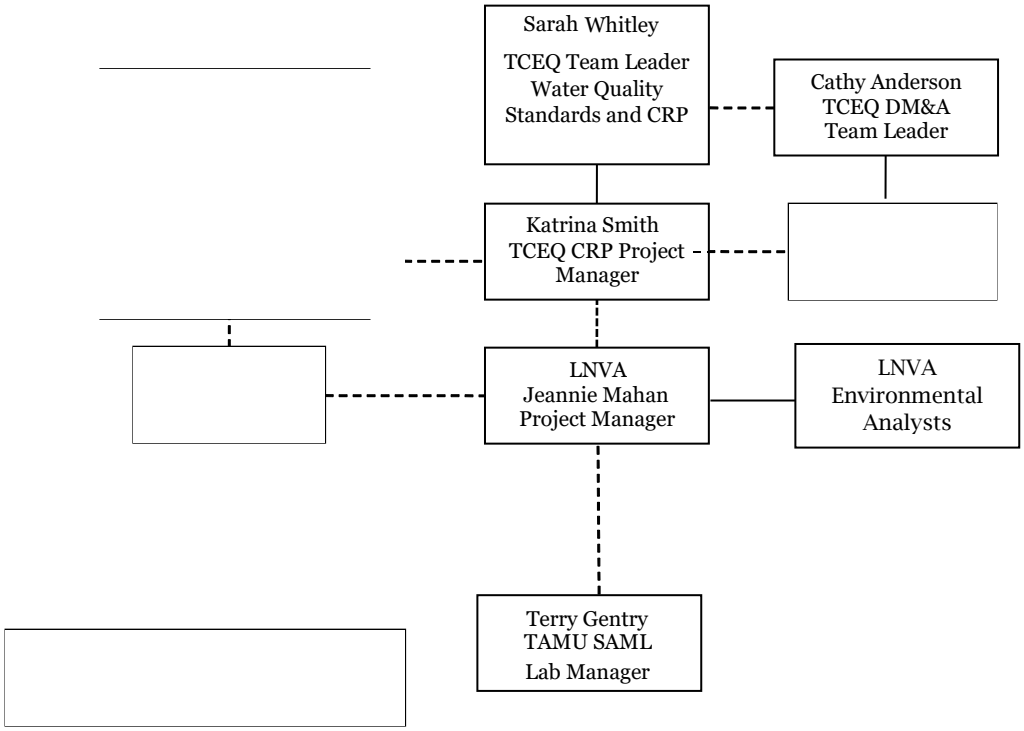
LNVA Environmental Analysts
Responsible for collecting data and water samples for bacteria source tracking special study. They will assist the LNVA Project Manager in ensuring the samples are delivered on time to TAMU soil and aquatic microbiology laboratory for analysis. Notifies LNVA Project Manager of any issues with the samples that may adversely affect the quality of data reported.

Texas A&M University (TAMU) Soil and Aquatic Microbiology Laboratory

Terry Gentry
Laboratory Manager
Responsible for accepting water samples from LNVA and performing the required analyses within their hold time. Responsible for analyzing bacterial source tracking data and sending a report to the LNVA Project Manager. Notifies the LNVA Project Manager of particular circumstances which may adversely affect the quality of data.

Project Organization Chart

Figure A4.1. Organization Chart – Lines of Communication



SS-A5 Problem Definition/Background

Segment 0607 Pine Island Bayou spans from the confluence with the Neches River in Hardin/Jefferson County to FM 787 in Hardin County. Pine Island Bayou is prolific with a heavy pine component throughout the segment. The only two major tributaries for Pine Island Bayou (PIB) are Little Pine Island Bayou, which flows from southeastern Polk County southeast through Hardin County to the Jefferson County line at Bevil Oaks, and Willow Creek, which originates northeast of Devers in Liberty County, flowing northeast for 15 miles before reaching its confluence with Pine Island Bayou in Jefferson County. Boggy Creek is a smaller tributary that flows from west of Lumberton to its confluence with PIB, south of Lumberton in Hardin County. Pine Island Bayou's drainage basin is made up of 657 square miles. The land making up the drainage basin is used primarily for timber, pastureland, agriculture, and oil and gas production. The upper reaches of the segment are heavily forested while the lower reaches provide drainage for the communities of Sour Lake, Pinewood Estates, Bevil Oaks, and northern Beaumont.

Pine Island Bayou was first placed on the 2008 Texas Integrated Report 303(d) List of Impaired Water Bodies for contact recreation due to elevated levels of *E. coli*. Little Pine Island Bayou, a tributary to Pine Island Bayou, was first listed for bacteria in 2006. Willow Creek was first listed in 2022. The Draft 2024 Texas Integrated Report-Potential Sources of Impairments and Concerns indicates additional data and information are needed before a management strategy is selected. Criteria limits in the Texas Surface Water Quality Standards are 126 colonies/100 mL of water for bacteria. The assessment results of Pine Island Bayou 0607_03 for bacteria yielded a geomean of 178.5 and 0607_04's geomean was assessed to be 127.96. Segment 0607A, Boggy Creek, has a geomean of 169.32. 0607B_01, Little Pine Island Bayou had a geomean of 195.94. Willow Creek, 0607C_01, had a geomean of 301.65. Twenty data points from 12/01/2015 to 11/30/2022 were assessed for the Draft 2024 Texas Integrated Report.

High concentrations of bacteria, which are found in both human and animal waste, may indicate a health risk to people who swim or wade in the water body. These activities are called "contact recreation" as described in the Texas Surface Water Quality Standards (TSWQS). The problem is often difficult to correct because the source of the contamination cannot be determined with certainty. Potential sources for the bacteria are currently listed as "non-point source" and "source unknown." A bacteria source tracking (BST) project is proposed as a way to better understand the sources of the impairment to assist in determining if a TMDL and subsequent Implementation Plan would be beneficial.
LNVA curr

LNVA currently monitors routine parameters at six stations in the Pine Island Bayou Watershed and TCEQ Region 10 monitors one station. In segment 0607, Pine Island Bayou at Hwy 69 station 10602, Pine Island Bayou at LNVA Pump Station, 10599, Pine Island Bayou at Sour Lake Rd, 10607, and Pine Island Bayou @105/770, 15367 are being monitored quarterly for routine parameters by LNVA. Boggy Creek, station 16127, is monitored for routine parameters by TCEQ Region 10. LNVA is also collecting 24 HR dissolved oxygen at several stations where special monitoring has been assigned. These sites include Pine Island Bayou

@69, 10602, Pine Island Bayou @ 105/770, 15367, Boggy Creek, 16127, and Willow Creek, 15345.

Preliminary plans for this study were presented at the 2024 LNVA Steering Committee meeting on 08/1/2024. Stakeholders expressed interest in the results of the study. No stakeholders were in opposition to the study.

SS-A6 Project/Task Description

The goal of this BST special study is to identify the major sources of bacterial loading in Pine Island Bayou Watershed. BST results can be broken down into three primary categories: bacteria from wildlife, bacteria from livestock and domesticated animals, and bacteria from humans.

The BST study design will consist of three sites in Segment 0607. The main branch of PIB was selected as well as two main tributaries that flow into Pine Island Bayou, Willow Creek and Little Pine Island Bayou. These are routine monitoring sites in the LNVA Clean Rivers Program not meeting the bacteria standards in the TSWQS. Recommendations for the *E. coli* impairments are to collect more information that will aid in future decision making for this waterbody. Upon approval of this special study appendix, one water sample will be collected quarterly by LNVA environmental analysts at each site – three events will target ambient conditions and one event will target stormwater conditions to understand bacterial loading during different environmental conditions. Ambient conditions consist of baseline flows with no recent rain events, and stormwater conditions follow a rain event with enough runoff to increase the USGS gauge 08041749 stage level three inches (+ or – 0.5) or higher. Water samples will be collected and delivered to the TAMU Soil and Aquatic Microbiology Laboratory (SAML) for processing. Each site will have 10 *E. coli* isolates analyzed. If stormwater conditions are not met during fiscal year 2025, additional studies may be conducted the following year and this special study appendix will be submitted to TCEQ with the fiscal year 2026-2027 Basin QAPP to extend the project into fiscal year 2026.

In addition to the water samples for *E. coli,* field and flow data will be collected as well. There can be strong correlations between the results collected for flow and bacteria. Field data and field observations can be helpful when assessing the data results to aid in drawing conclusions about the cause of the impairment.

Amendments to the QAPP

Amendments to the Special Study Appendix may be necessary to address incorrectly documented information or to reflect changes in project organization, tasks, schedules, objectives, and methods. Requests for amendments will be directed from the LNVA Project Manager to the CRP Project Manager electronically. Amendments are effective immediately upon approval by the LNVA Project Manager, the LNVA QAO, the TCEQ CRP Project Manager, the TCEQ CRP Lead QA Specialist, the TCEQ CRP Project QA Specialist, the TCEQ CRP Team Leader, the TCEQ DM&A Team Leader, and additional parties affected by the amendment. Amendments are not retroactive. No work shall be implemented without an approved Special Study Appendix or amendment prior to the start of work. Any activities under this contract that commence prior to the approval of the governing QA document constitute a deficiency and are subject to corrective action as described in section C1 of the basin-wide QAPP. Any deviation or deficiency from this QAPP that occurs after the execution of this QAPP should be addressed through a Corrective Action Plan (CAP). An amendment may be a component of a CAP to prevent future recurrence of a deviation. Amendments will be

incorporated into the QAPP by way of attachment and distributed to personnel on the distribution list by the LNVA Project Manager.

SS-A7 Quality Objectives and Criteria

The objective of this short-term monitoring project will be to use bacterial source tracking to determine the sources of bacterial loading in the Pine Island Bayou Watershed. The data collected during this proposed special study (CRP Task 7) will not be entered into SWQMIS or assessed in the Integrated Report. The data will be used as a preliminary step to address the impairment by (1) educating stakeholders and (2) providing better information to develop a management strategy.

Information gathered from this BST study will be shared with stakeholders in the 2026 Basin Summary Report and Steering Committee Meeting. This study could assist in leading to the development of a TMDL and I-plan with the ultimate goal being that the waterbodies eventually meet contact recreation use criteria as defined in the TSWQS.

Water sampling will be conducted consistent with the most recent version of TCEQ's *Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods, 2012* (RG-415). Water samples collected will be transported to SAML for bacteria isolation and BST analysis. Methods outlined in Tables SS-A7.1 and SS-B2 will be employed. Appropriate DQOs, performance criteria, and QA/QC requirements for this analysis are also reported in Tables SS-A7.1 and SS-B2.

The measurement performance specifications to support the project objectives are specified in Table SS-A7.1.

Table SS-A7.1 — Measurement Performance Specifications

Parameter	Units	Matrix	Method	AWRL	Precision of Laboratory Duplicates	Accuracy[1]	Lab
Field Parameters							
TEMPERATURE, WATER (DEGREES CENTIGRADE)	DEG C	water	SM 2550 B and TCEQ SOP V1	NA	NA	NA	Field
PH (STANDARD UNITS)	s.u.	water	EPA 150.1and TCEQ SOP V1	NA	NA	NA	Field
OXYGEN, DISSOLVED (MG/L)	mg/L	water	SM 4500-O G and TCEQ SOP, V1	NA	NA	NA	Field
SPECIFIC CONDUCTANCE, FIELD (US/CM @ 25C)	us/cm	water	EPA 120.1and TCEQ SOP V1	NA	NA	NA	Field
PRESENT WEATHER (1=CLEAR,2=PTCLDY,3=CLDY,4=RAIN, 5=OTHER)	NU	other	NA	NA	NA	NA	Field
DAYS SINCE PRECIPITATION EVENT (Days)	days	Other	TCEQ SOP V1	NA	NA	NA	Field
Flow Parameters							
FLOW STREAM, INSTANTANEOUS (CUBIC FEET PER SEC)	cfs	water	TCEQ SOP V1	NA	NA	NA	Field

FLOW SEVERITY:1=No Flow,2=Low,3=Normal,4=Flood,5=High,6=Dry	NU	water	TCEQ SOP V1	NA	NA	NA	Field
STREAM FLOW ESTIMATE (CFS)	cfs	water	TCEQ SOP V1	NA	NA	NA	Field
FLOW MTH 1=GAGE 2=ELEC 3=MECH 4=WEIR/FLU 5=DOPPLER	NU	other	TCEQ SOP V1	NA	NA	NA	Field
Bacteria Source Tracking Parameters							
E. coli in water, isolation, mTEC	cfu/100 mL	water	EPA 1603 (membrane filter culture on modified mTEC agar)	1 cfu[2]	NA	NA	SAML
E. coli RiboPrint fingerprint	NA	water	UT Health SOP (DNA/ image matching); section C-6	NA	90 % identical[3]	90% correct[3]	SAML
E. coli ERIC-PCR fingerprint	NA	water	UT Health SOP (DNA/ image matching); section C-5	NA	90 % identical[3]	90% correct[3]	SAML

[1]Manufacturer specifications are presented for accuracy limits.
[2]Minimum detection limits for field parameters represent manufacturer specifications and will be used for the AWRL in this instance.
[3]Accuracy and laboratory method precision for BST will be determined using an *E. coli* QC isolate and DNA from known-source samples.

References:

United States Environmental Protection Agency (USEPA) "Methods for Chemical Analysis of Water and Wastes," Manual #EPA-600/4-79-020
American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), "Standard Methods for the Examination of Water and Wastewater," 24th Edition, 2022.
TCEQ SOP - Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods, 2012.
American Society for Testing and Materials (ASTM) Annual Book of Standards, Vol. 11.02

Ambient Water Reporting Limits (AWRLs)

Minimum detection limits for field parameters represent manufacturer specifications and will be used for the AWRL in this instance.

Precision

RiboPrinting and ERIC-PCR BST are qualitative assays, that generate two different types of DNA fingerprints. The precision of the ERIC-PCR and RiboPrinting can be measured as the percent of *E. coli* isolates that, when typed multiple times, produce the same ultimate source result in terms of the source identified. Precision for ERIC-PCR and RiboPrinting will be determined using a laboratory control strain of *E. coli* (QC101). For ERIC-PCR and RiboPrinting, the data quality objective is 90% precision.

Bias

As described in Section A7 of the FY 24–25 basin-wide QAPP.

Representativeness

Representativeness is the extent to which the measurements actually represent the true environmental conditions that are being measured. Bacterial source tracking samples will be collected during ambient and stormwater conditions (if stormflows occur during project time length) at three different sites. (Note: If stormflows do not occur before the end of the project time, additional supplemental sampling may possibly be conducted in FY26 and encompassed in the future FY26 – 27 Basin QAPP, pending TCEQ discussion and approval.) The BST analyses will identify source(s) of *E. coli* representative to the environmental conditions and site locations the samples were collected.

Accuracy

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

Accuracy for BST methods will be assessed using the *E. coli* (QC101) lab control strain for *E. coli* RiboPrint Fingerprinting (ERIC-RP) and *E. coli* isolate DNA from known human and animal sources for *E. coli* library-independent PCR. For ERIC-RP, the data quality objective is 90% accuracy for correct identification to library strain.

Comparability

As described in Section A7 of the FY 24–25 basin-wide QAPP.

Completeness

As described in Section A7 of the FY 24–25 basin-wide QAPP.

SS-A8 Special Training/Certification

As described in section A7 of the basin-wide QAPP. In addition, BST analytical capabilities will be maintained by SAML.

SS-A9 Documents and Records

Document/Record	Location	Retention	Format*
QAPPs, amendments, and appendices	LNVA, SAML	5 years	Electronic
Field SOPs	LNVA	5 years	Electronic
Laboratory SOPs	LNVA	5 years	Electronic
	SAML	2 years	
QAPP distribution documentation	LNVA	5 years	Electronic
Field staff training records	LNVA	5 years	Electronic
Field equipment calibration/maintenance logs	LNVA	5 years	Electronic
Field Instrument printouts	LNVA	5 years	Electronic
Field data sheets	LNVA	5 years	Electronic/Paper
Chain of custody records	LNVA	5 years	Electronic/ Paper
	SAML	2 years	
Laboratory calibration records and maintenance logs	LNVA	5 years	Electronic
	SAML	2 years	
Laboratory instrument printouts	LNVA	5 years	Electronic
Laboratory data reports/ results	LNVA/SAML	5 years	Electronic/Paper
Corrective Action Documentation	LNVA	5 years	Electronic/Paper
Sheets of Lading for Fecal Specimens	SAML	2 years	Electronic/Paper
Bacteriological Data Sheet	SAML	2 years	Electronic/Paper
Quarterly progress reports/final report/data**	LNVA	5 years	Electronic/Paper

**Formats of electronic/paper can be either/or.*
***Final report will follow the format described in A9 of the FY24/25 Basin QAPP.*

SS-B1 Sampling Process Design

The data collection design is summarized in Table SS-B1.1 (Sampling Sites and Monitoring Frequencies) and Figure SS-B1 (Sample Site Maps).

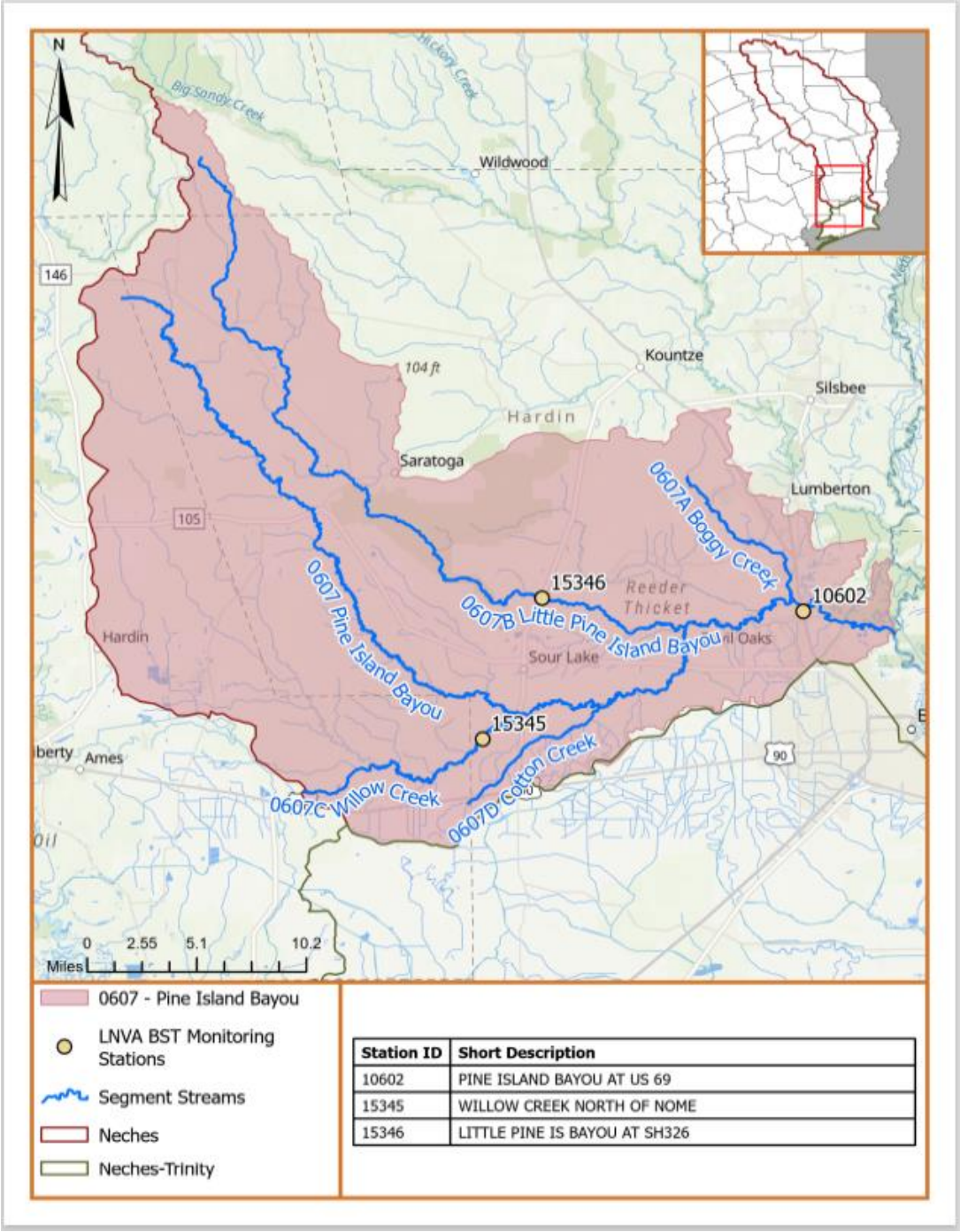
Table SS-B1.1 Sample Design and Schedule, FY 2025

Site Description*	Station ID	Waterbody ID	Region	SE	CE	BST[1]	Flow	Field	Comments
PINE ISLAND BAYOU AT US 69 /US 96/US 287 AT VOTH	10602	0607	10	LV	LV	4	4	4	USGS Station 08041749 for flow.
LITTLE PINE ISLAND BAYOU AT SH 326 5.68 KM NORTH OF CITY OF SOUR LAKE	15346	0607B	10	LV	LV	4	4	4	Doppler flow method to be used.
WILLOW CREEK AT UNNAMED ROAD 4.87 KM NORTH NORTHWEST OF NOME 6.78 KM UPSTREAM OF PINE ISLAND BAYOU CONFLUENCE/SH 326	15345	0607C	10	LV	LV	4	4	4	Doppler flow method to be used.

[1]The 4 BST samples consist of 3 ambient and 1 stormwater sample.

Figure SS-B1. Sampling Site Map

Maps of stations monitored by the LNVA are provided below. The maps were generated by the LNVA. This product is for informational purposes and may not have been prepared for or be suitable for legal, engineering, or surveying purposes. It does not represent an on-the-ground survey and represents only the approximate relative location of property boundaries. For more information concerning this map, contact Jeannie Mahan at jeanniem@LNVA.dst.tx.us.



Sample Design Rationale and Site Selection Criteria

The sample design rationale is based on the intent of the study to identify sources of *E. coli* within the impaired section of the Pine Island Bayou watershed during ambient and stormwater conditions. Water samples from 3 sites will be collected a total of 4 times. These samples will be collected once a quarter. The number of sites selected was based on the total funding for the project while the location of the sites was based on providing a representation across the impaired segments and ease of site access. The sites selected are part of LNVA's CRP routine monitoring in Segment 0607 Pine Island Bayou. Site 10602 is representative of the main branch of Pine Island Bayou and where all the smaller tributaries have drained. 15345 Willow Creek and 15346 Little Pine Island Bayou are two major tributaries that flow into Pine Island Bayou and a way to separate out the sources of bacterial loading to individual tributaries. Section SS-A6 Project/Task Description provides additional information regarding sample design

SS-B2 Sampling Methods

Field Sampling Procedures

The field sampling procedures for this special study will be identical to collecting *E. coli* in the FY 24–25 basin-wide QAPP.

Sample volume, container types, minimum sampling volume, preservation requirements, and holding time requirements

Bacterial source tracking uses the container, preservation requirements, and holding time for bacteriological sampling as listed below in Table SS-B2. Certificates from sample container manufacturers are maintained by LNVA. LNVA will purchase new, pre-cleaned containers for the BST project. Sample containers are not reused but are properly disposed of after use.

Table SS-B2. Sample Storage, Preservation, and Handling Requirements

Parameter	Matrix	Container	Preservation	Sample Volume	Holding Time
E. coli, mTEC	Water	Sterile, Polyethylene	Sodium thiosulfate; Ice; <6 ˚C not frozen	290 mL	24 hours

Sample Containers

E. coli samples for BST will use 290 mL bottles from IDEXX which contain 1% sodium thiosulfate to neutralize residual chlorine up to 15 mg/L. The 290 mL container was selected based on the *Texas Bacterial Source Tracking Program (FY20)* QAPP sampling methods using sterile 200 mL Whirl-Pak for *E. coli* collection. The TSSWCB Bacterial Source Tracking Program (FY20) QAPP can be found here:

https://tsswcb.texas.gov/sites/default/files/files/programs/nonpoint-source-managment/20-52-qapp-bst2020-1-10-2022.pdf

Processes to Prevent Contamination

As described in Section B2 of the FY24–25 basin-wide QAPP.

Documentation of Field Sampling Activities

Field sampling activities will be documented on a form that will include station/site ID, location, sampling time, date, depth, sampler's name, field/flow information, and detailed observational information. This form can be found in SS-Appendix A.

Recording Data

As described in Section B2 of the basin-wide QAPP.

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

As described in Section B2 of the FY24–25 basin-wide QAPP.

SS-B3 Sample Handling and Custody

Sample Labeling

Samples from the field are labeled on the container, or on a label, with an indelible marker. Label information includes:

- Site identification
- Date and time of collection
- Preservative added
- Sample type (i.e., analyses) to be performed

Sample Handling

Chain of Custody (COC) forms will be included and referenced. The COC form can be found in SS-Appendix B. Information will include date and time of collection, site identification, sample matrix, number of containers, preservative used, analyses required, name of collector, custody transfer signatures, and the shipping bill, if applicable, will be included on the form. LNVA staff will collect the samples and transport directly to the TAMU Soil and Aquatic Microbiology Laboratory in College, Station, TX within 24 hours. The samples will be transported on ice.

Sample Tracking Procedure Deficiencies and Corrective Action

As described in Section B3 of the FY24–25 basin-wide QAPP.

SS-B4 Analytical Methods

The analytical methods, associated matrices, and performing laboratories are listed in Table SS-A7.1 of section SS-A7. The authority for analysis methodologies under CRP is derived from the 30 Tex. Admin. Code ch. 307, in that data generally are generated for comparison to those standards and/or criteria. The Standards state “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 as amended, 40 CFR 136, or other reliable procedures acceptable to the TCEQ, and in accordance with chapter 25 of this title.” Copies of laboratory SOPs are retained by the SAML and are available for review by the TCEQ. Laboratory SOPs are consistent with EPA requirements, as specified in the method. A copy of the BST Standard Operating Procedure has been included in Appendix C of this document.

Standards Traceability

As described in Section B4 of the FY24–25 basin-wide QAPP.

Analytical Method Deficiencies and Corrective Actions

As described in section B4 of the FY24–25 basin-wide QAPP.

SS-B5 Quality Control

Sampling Quality Control Requirements and Acceptability Criteria

No field QC samples will be collected for this special study.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

Method specific QC requirements

QC samples 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 a laboratory control sample (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 SS-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. It is the responsibility of the LNVA QAO to verify that the data are representative. The LNVA QAO 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.

Positive controls

Positive controls will consist of a laboratory control strain of *E. coli* (QC101) for ERIC-PCR and RiboPrinting and will be included in every preparation batch. For PCR-based library independent BST methods positive controls will consist of fecal DNA or *E. coli* isolate DNA from known human and animal sources and will be included with every preparation batch. Positive controls should always test positive. Samples associated with a failed positive control 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. The analytical methods are listed in Table SS-A7.1 of Section A7. Detailed SOPs for these methods are provided in Appendix C as an attachment to this special study QAPP.

Quality Control or Acceptability Requirements Deficiencies and Corrective Actions

Notations of blank contamination will be noted in the QPR. Corrective action will involve identification of the possible cause of the contamination failure, where possible. 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 LNVA in the QPR. Corrective Action Reports will be maintained by the LNVA Project Manager.

SS-B6 Instrument/Equipment Testing, Inspection, and Maintenance

All sampling equipment testing and maintenance requirements are detailed in the SWQM Procedures. Equipment records are kept on all field equipment and a supply of critical spare parts is maintained. All LNVA laboratory tools, gauges, instrument, and equipment testing and maintenance requirements are contained within LNVA laboratory QM(s).

All SAML 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 LNVA.

Failures in any testing, inspections, or calibration of equipment will result in a corrective action report (CAR) and resolution of the situation will be reported to the LNVA. CARs will be maintained by the LNVA project Manager.

SS-B7 Instrument Calibration and Frequency

LNVA field equipment calibration requirements are contained in the SWQM Procedures. Post-calibration check error limits and the disposition resulting from errors are adhered to. Data collected from field instruments that do not meet the post-calibration check error limits specified in the SWQM Procedures will not be used in this special study.

All SAML 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 SAML instruments or devices used in obtaining environmental data will be used according to appropriate laboratory or field practices. Written copies of SAML SOPs are available for review upon request. Standards used for SAML 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 SAML testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the LNVA Project Manager. CARs will be maintained by the LNVA Project Manager.

SS-B8 Inspection/Acceptance of Supplies and Consumables

For LNVA, there are no special requirements for acceptance are specified for field sampling supplies and consumables. Laboratory QMs contain information for inspection/acceptance of laboratory-related supplies and consumables. Certification and traceability documentation are recorded in the LIMS system.

For the SAML, 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.

SS-B9 Acquired Data

USGS gage station data will be used throughout this project to aid in determining gage height and flow. QA checks are completed on gage data by the USGS and the data are approved by the USGS and permanently stored at the USGS. This data will be submitted with the final BST report.

SS-B10 Data Management

LNVA Field Data

Field water quality data are generated by the LNVA environmental analysts on field sheets. The LNVA Data Manager inserts received data into LNVA's LIMS database and performs automated checks of the data using in-house screening tools and adds unique tag identification numbers to the data. The field data are combined with the BST data into a final BST report. The TCEQ project manager reviews the data and associated report and provides comments or asks for clarification.

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. The LNVA 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 Leader 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 SAML. SAML will create a report for each batch of samples received. This report includes a pie chart breakdown for each sample by source and also a table with the ID for each *E. coli* isolate tested. LNVA will also keep a permanent copy of the data on LNVA's server in the Task 7 special studies folder for FY 24–25.

SS-C1 Assessments and Response Actions

LNVA is responsible for monitoring the status and records of the projects to ensure requirements are being fulfilled. Equipment testing and data completeness will be conducted as needed by SAML to determine passing/failing of equipment for repair or replacement and an assessment of the sample analysis that may require them be reanalyzed.

Corrective Action

LNVA Project Manager is responsible for analysts implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the LNVA Project Manager. 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.

SS-C2 Reports to Management

Reports to Planning Agency Project Management

As described in Section C2 of the FY24–25 basin-wide QAPP.

Reports to TCEQ Project Management

As described in Section C2 of the FY24–25 basin-wide QAPP.

Reports by TCEQ Project Management

As described in Section C2 of the FY24–25 basin-wide QAPP.

SS-D1 Data Review, Verification, and Validation

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 SS-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 SS-D2, below. The LNVA is responsible for validating that all data collected meet the DQOs of the project are suitable for submission in the final report to the TCEQ.

SS-D2 Verification and Validation 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 LNVA Project Manager 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. The data 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 performer 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 performer consults with the LNVA Project Manager to establish the appropriate course of action, or the data associated with the issue are rejected.

The LNVA Project Manager and SAML 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 TCEQ.

SS-D3 Reconciliation with User Requirements

Data produced in this project will be analyzed and reconciled with project data quality requirements. Data which do not meet requirements will not be considered appropriate for any of the uses noted in Section SS-A6 and SS-A7.

ATTACHMENT 1 Example Letter to Document Adherence to the QAPP Appendix G

DATE: date
TO: name

organization

FROM: name

organization

RE: Appendix G to the LNVA Fiscal Year 2024–2025 CRP QAPP

Please sign and return this form by date to:

(address)

I acknowledge receipt of the "Appendix G of QAPP Title, Revision Date". I understand the document(s) describe quality assurance, quality control, data management and reporting, and other technical activities that must be implemented to ensure the results of work performed will satisfy stated performance criteria. My signature on this document signifies that I have read and approved the document contents pertaining to my program. Furthermore, I will ensure that all staff members participating in CRP activities will be required to familiarize themselves with the document contents and adhere to them as well.

_____

Name Date

Copies of the signed forms should be sent by the LNVA to the TCEQ CRP Project Manager within 60 days of TCEQ approval of the QAPP.

SS-Appendix A: Field Data Sheet

	Lower Neches Valley Authority			
	Bacteria Source Tracking Field Sheet			
Collectors:				
Container	Sterile; Polyethylene (290 mL)			
Preservation:	Sodium thiosulfate; Ice; < 6 ° C not frozen			
		Field Parameters		
Station Location				
Collection Date				
Collection Time				
Dissolved Oxygen				
Temperature				
pH				
Conductivity				
End Depth				
Days Since Last Precipitation				
Flow Severity [1]				
Present Weather [2]				
Cattle [3]				
Bird Rookery [3]				
Wild Hog [3]				
Instantaneous Flow				
Flow Method [4]				
[1] **1= No Flow 2= Low 3= Normal 4=Flood 5= High 6= Dry**				
[2] **1= Clear 2= Partly Cloudy 3=Cloudy 4=Rain 5= Other**				
[3] **1= Observed 2= Evidence 3= Not Observed**				
[4] **1= Gauge 2= Electronic 3= Mechanical 4= Weir/Flume 5= Doppler**				
Notes:				

SS-Appendix B: Chain of Custody Form

Lower Neches Valley Authority Bacteria Source Tracking Chain of Custody
Name of Collectors:
Parameters: Bacteria Source Tracking
Matrix: Water
Container: Sterile; Polyethylene
Preservation: Sodium Thiosulfate; Ice; < 6 C not frozen
Sample Volume: 290 mL
Holding Time: 8 Hours

Sample Information			
Station Location			
Collection Date			
Collection Time			

Lab Analyses Required
E. coli in water, isolation, mTEC
E. coli RiboPrint fingerprint
E. coli ERIC-PCR fingerprint

Transfers	Relinquished By	Date/Time	Received By	Data/Time

SS-Appendix C: BST Standard Operating Procedures

C-3: Cultivation of *E. coli* from Water Samples and Pre-Processing for Isolation and Bacterial Source Tracking

August 13, 2018

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 initial growth and pre-processing of *E. coli* from water samples for transport to Bacterial Source Tracking Laboratories (BST) for further *E. coli* isolation and characterization for BST analyses.

2.0. SUMMARY OF THE METHOD

Water samples are processed with EPA Method 1603 to grow and enumerate *E. coli* on solid media. Plates containing *E. coli* colonies are then transported/shipped to a BST Laboratory for subsequent analyses.

3.0. HEALTH AND SAFETY WARNINGS

Water samples may contain pathogenic microorganisms. The analyst should treat all such samples 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.

4.0. 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.

5.0. PERSONNEL QUALIFICATIONS

This SOP is written for persons with a thorough knowledge of laboratory and microbiological procedures, especially aseptic technique.

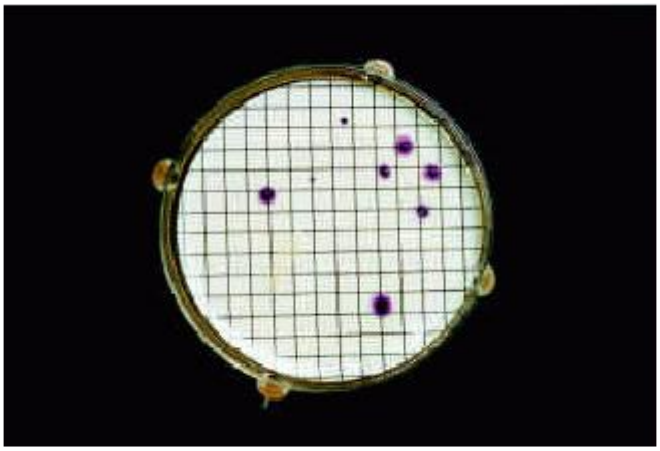
6.0. EQUIPMENT AND SUPPLIES

6.1 Pipettes, sterile, plastic, of appropriate volume
6.2 Sterile graduated cylinders, 100 mL, covered with aluminum foil.

6.3 Sterile membrane filtration units (funnel), plastic, wrapped with aluminum foil. Sterile membrane filtration units (funnel), plastic, in individual autoclave bag or covered with aluminum foil.
6.4 Electric vacuum pump, as a vacuum source
6.5 Filter flask, vacuum, usually 1.0 L, with appropriate tubing
6.6 Forceps, straight or curved, with smooth tips to handle filters without damage
6.7 Ethanol, wide-mouth container, for flame-sterilizing forceps
6.8 Whirl-Pak® bags or equivalent
6.9 Autoclave or steam sterilizer capable of achieving 121°C [15 lb pressure per square inch (PSI) for 15 minutes
6.10 Burner for sterilizing loops and needles
6.11 Modified mTEC agar plates
6.12 Membrane filters, sterile, white, grid marked, 47 mm diameter, with 0.45 µm pore size (Cat#HAWG047S6)
6.13 Incubator maintained at 35°C ± 0.5°C
6.14 Waterbath maintained at 44.5°C ± 0.2°C
6.15 Filter paper
6.16 Marker
6.17 Parafilm
6.18 Cooler with ice or blue ice for transport of samples
6.19 Refrigerator (~4°C)

7.0. PROCEDURAL STEPS

7.1. Follow the EPA Method 1603 Modified mTEC procedure (EPA-821-R-09-007;



http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/method_1603.pdf).

7.2. After 22 +/- 2 hour incubation at 44.5°C, red or magenta colonies are considered 'typical' *E. coli*.

7.3. Using a black Sharpie or similar marker, mark *E. coli* colonies with a 'dot' on the back of the plate. This helps to ensure that colonies which grew during the incubation period, as opposed to during shipping or storage, are subsequently isolated. If the

colonies were counted, please also write the total number of counted colonies on the back of each plate.

7.4. After incubation and counting, immediately store plates at 4ºC 'media-side up' (i.e., upside down), so condensation does not fall onto the filter during storage.

7.5. The plates should be shipped as soon as possible (preferably the day after filtration, but no later than three days following filtration) to the BST Laboratory (addresses below) via overnight delivery.

7.6. In preparation for shipment, each plate should be sealed with Parafilm around the edge to protect the cultures from contamination during transit. Dilution series for each sample should subsequently be grouped together and placed in secondary containers such as large Whirl-Pak or zip-top bags.

7.7. 'Blue-ice' or freezer blocks should be used to keep the plates cool (~4ºC), but not frozen during transport. Do not use dry ice for shipment as this will freeze the media and cultures.

7.8. Ship plates (and COCs) in insulated coolers with sufficient ice packs to maintain ~4°C to:

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

7.9. Notification of shipment should be sent to the appropriate lab via email or phone (see contact info below) no later than the day of overnight shipping. Notification should include the *E. coli* count datasheet (if available), shipment tracking number, and direct contact person for confirmation upon receipt of samples.

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

SAML

Terry Gentry; tjgentry@tamu.edu; 979-845-5323

8.0. QUALITY ASSURANCE AND QUALITY CONTROL

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

USEPA. 2009. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC). EPA-821-R-09-007. December 2009.

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 contact information
3	March 2019	Anna Gitter/Elizabeth Casarez	Updated UTH laboratory contact information
4	June 2020	Anna Gitter	Updated UTH laboratory contact information
5	August 2022	Anna Gitter	Updated UTH laboratory address

C-4: Archival of *E. coli* Isolates

August 13, 2018

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 isolation and archival of *E. coli* isolates for further Bacterial Source Tracking (BST) analyses.

2.0. SUMMARY OF THE METHOD

Individual *E. coli* colonies are selected from EPA Method 1603 plates, streaked onto nutrient agar-MUG plates for verification and placed into glycerol stocks for archival at -80°C.

3.0. HEALTH AND SAFETY WARNINGS

Environmental *E. coli* isolates may be pathogenic. Water samples may contain pathogenic microorganisms. All handling of cultures will be performed using a Class II biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. Analysts should wear appropriate personal protective equipment (e.g., gloves). Analysts should wear eye protection and exercise caution when using UV light to examine plates.

4.0. INTERFERENCES

It is important that the isolates be streaked to purity in order to prevent issues arising from co-cultures. The archived isolates should be from colonies which have been plated for purity several times and lab personnel are confident that purity has been achieved.

5.0. PERSONNEL QUALIFICATIONS

This SOP is written for persons with a thorough knowledge of laboratory and microbiological procedures, especially aseptic technique.

6.0. EQUIPMENT AND SUPPLIES

- 6.1 Sterile, plastic inoculating loops or needles; alternatively, a wire loop or needle can be used if sterilized between transfers
- 6.2 Nutrient agar-MUG plates
- 6.3 Brain Heart Infusion (BHI) agar plates
- 6.4 Longwave UV lamp
- 6.5 Sterile cryovials (2 ml)
- 6.6 Liquid nitrogen in dewar vessel
- 6.7 Sterile, tryptone soy broth (TSB) containing 20% reagent grade glycerol
- 6.8 Vortex
- 6.9 Pipette and sterile tips (1 ml)
- 6.10 Incubator (~35°C)

6.11 Freezer (-80°C)
6.12 Class II biosafety cabinet

7.0. PROCEDURAL STEPS

7.1 Select a presumptive *E. coli* colony from the EPA Method 1603 plates used to process tested water samples. Streak cells from the colony onto a NA-MUG plate. Depending upon the number of isolates desired, repeat the process streaking each isolate onto an individual NA-MUG plate.

7.2 Incubate NA-MUG plates at ~35°C for ~24 hr.

7.3 Examine the plates using a long-wave handheld UV lamp. *E. coli* colonies will fluoresce.

7.4 If a culture is verified as *E. coli* in the above step and appears to be pure based on uniform appearance on the NA-MUG plate, select a well-isolated colony using a bacteriological loop and streak onto a Brain Heart Infusion (BHI) agar plate. Incubate the plate at ~35°C for ~24 hr. If the culture is not pure, continue streaking individual colonies onto NA-MUG until purity is achieved.

7.5 Select a well-isolated colony using a bacteriological loop and transfer the colony to a labeled, sterile cryovial containing 1 mL of tryptone soy broth (TSB) with 20% reagent grade glycerol.

7.6. Once the colony has been transferred to the cryovial, firmly cap the cryovial and verify that the cells have been resuspended by vortexing for several seconds.

7.7. Plunge the cyrovial into liquid nitrogen until frozen. Immediately transfer to a cryostorage box and place in -80°C freezer. Cultures may be stored for several years under these conditions.

7.8. 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. 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 hr. Reclose the cryovial before the contents thaw and return it to the -80°C freezer.

8.0. QUALITY ASSURANCE AND QUALITY CONTROL

A positive control (*E. coli* QC101) is processed with each batch of 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.

USEPA. 2009. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC). EPA-821-R-09-007. December 2009.

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
3	August 2022	Anna Gitter	Updated UTH Lab name

C-5: DNA fingerprinting of *E. coli* Isolates using enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR

August 13, 2018

Joy Truesdale
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 DNA fingerprinting *E. coli* isolates using enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR.

2.0. SUMMARY OF THE METHOD

Cell suspensions from individual *E. coli* isolates are DNA fingerprinted using ERIC-PCR. Following PCR, amplicons are analyzed using gel electrophoresis to generate the DNA fingerprint which will ultimately be used for further Bacterial Source Tracking (BST) analysis.

3.0. HEALTH AND SAFETY WARNINGS

Environmental *E. coli* isolates may be pathogenic. Water samples may contain pathogenic microorganisms. All handling of live cultures will be performed using a Class II biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. Ethidium bromide is a mutagen and should be handled with care. Analysts should wear appropriate personal protective equipment (e.g., gloves). Analysts should wear eye protection and exercise caution when using UV light to image gels.

4.0. INTERFERENCES

It is important that the isolates be streaked to purity in order to prevent issues arising from co-cultures. The tested isolates should be from colonies which have been plated for purity several times and lab personnel are confident that purity has been achieved.

5.0. PERSONNEL QUALIFICATIONS

This SOP is written for persons with a thorough knowledge of laboratory and microbiological procedures, especially aseptic technique, PCR, and gel electrophoresis.

6.0. EQUIPMENT AND SUPPLIES

6.1 Sterile, plastic inoculating loops
6.2 Sterile, 1.5 ml microfuge tubes
6.3 Sterile, molecular-grade water
6.4 Vortex
6.5 *E. coli* QC101 cell-suspension
6.6 Thermal cycler
6.7 Pipettes and sterile tips (5-1000 µl)
6.8 PCR master mix (recipe below)
6.9 Agarose

6.10 1X Tris/Borate/EDTA (TBE)
6.11 Microwave
6.12 Electrophoresis system with recirculating pump
6.13 Ziploc bags
6.14 Freezer (-20°C)
6.15 6X ERIC-PCR loading buffer
   a. 25 mg bromphenol blue (0.25%)
   b. 1.5 g ficoll 400 (15%)
   c. Add molecular grade water to 10 mL
   d. Divide into 1 mL aliquots and freeze
   e. The aliquot currently being used can be stored in the cold room or at 4°C
6.16 100 bp ladder (0.33 µg/10 µL) (1500 µL final, enough for 150 lanes)
   a. 200 µL Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µL 100 bp ladder
   b. 300 µL 6X ERIC-PCR loading buffer
   c. 150 µL 10X PCR buffer
   d. 850 µL molecular grade water
   e. Store in cold room
6.17 Ethidium bromide stain (0.5 µg/mL)
   a. 1250 mL 1X TBE
   b. 62.5 µL ethidium bromide (Sigma, 10 mg/mL)
   c. Store covered at room temp,
   d. Can use up to 5 times by adding 10 µL ethidium bromide each additional use
6.18 ERIC-PCR blank;
   a. 100 µL 10X PCR buffer
   b. 200 µL 6X ERIC-PCR loading buffer
   c. 900 µL molecular grade water
   d. Store in cold room or at 4°C
6.19 Class II biosafety cabinet
6.20 PCR plates
6.21 Platform shaker
6.22 Gel imager
6.23 Cold room (~4°C)

7.0. PROCEDURAL STEPS

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

7.2. 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.

7.3. 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 thermal 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 (µL)	Final Calc	Final Units
Molecular Grade Water	**819**		
10X PCR buffer I w Mg (Life Technologies)	**130**	1	X (1.5 mM)
20 mM dNTP (GE Healthcare)	**13**	200	µM each
ERIC Primer Mix*	**130**	600	nM each
BSA (30 mg/ml)	**65**	1.5	µg/µL
AmpliTaqGold (Life Technologies)	**13**	2.5	Units/rxn

*ERIC1R 5' ATGTAAGCTCCTGGGGATTCAC;
ERIC2 5' AAGTAAGTGACTGGGGTGAGCG

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

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

7.6. Carefully seal plate using an adhesive PCR cover.

7.7. 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

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

7.9. 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.

7.10. 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 containing pre-cooled 1X TBE buffer. Replace TBE in gel tank after it has been used twice.

7.11. Remove PCR reactions from freezer. Add 10 µL of 6X ERIC-PCR Loading Buffer to each PCR well and mix with pipette tip.

7.12. 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.

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

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

7.15. After electrophoresis, stain gel in Ethidium Bromide Stain for 20 minutes with rocking on a platform shaker (save stain, see Step 6.17d).

7.16. Destain gel for 10 minutes in 1X TBE buffer. Save destaining solution. Discard after three uses.

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

8.0. QUALITY ASSURANCE AND QUALITY CONTROL

A method blank (sterile water; “no template control”) and positive control (*E. coli* QC101) is processed with each batch of 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	Joy Truesdale	Initial Release
2	August 2018	Lucas Gregory	Update UTH Lab name
3	August 2022	Anna Gitter	Updated UTH Lab name

C-6: DNA fingerprinting of *E. coli* Isolates using the DUPONT Riboprinter® system

August 13, 2018

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1.0. PURPOSE AND APPLICABILITY

The purpose of this Standard Operating Procedure (SOP) is to establish a uniform procedure for DNA fingerprinting *E. coli* isolates using the DuPont RiboPrinter® System.

2.0. SUMMARY OF THE METHOD

Cell suspensions from individual *E. coli* isolates are DNA fingerprinted using the DuPont RiboPrinter® System. The DuPont™ RiboPrinter® System automates restriction fragment length polymorphism (RFLP) analysis and targets the rRNA-coding region of the bacterial genome. Restriction enzymes cut bacterial DNA into fragments that are processed to form a characteristic banding pattern or "fingerprint." The system captures an image of the banding pattern and digitizes it as a RiboPrint™ pattern. This pattern is ultimately compared to a reference database of patterns for further Bacterial Source Tracking (BST) analysis.

3.0. HEALTH AND SAFETY WARNINGS

Environmental *E. coli* isolates may be pathogenic. All handling of live cultures will be performed using a Class II biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. Analysts should wear appropriate personal protective equipment (e.g., gloves).

4.0. INTERFERENCES

It is important that the tested isolates be streaked to purity in order to prevent issues arising from co-cultures. The tested isolates should be from colonies which have been plated for purity several times and lab personnel are confident that purity has been achieved.

5.0. PERSONNEL QUALIFICATIONS

This SOP is written for persons with a thorough knowledge of laboratory and microbiological procedures, especially aseptic technique.

6.0. EQUIPMENT AND SUPPLIES

6.1 DuPont RiboPrinter® System and consumables
6.2 *Hin*dIII restriction enzyme 50 U/µL working stock (*Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as follows. 50 U/µL: 26.5 µL *Hin*d III and 26.5 µL of NEB 10X Buffer 2.1.
6.3 BHI agar plates
6.4 Incubator (37°C)
6.5 Sterile, plastic inoculating loops or needles
6.6 Sterile, microcentrifuge tubes
6.7 Pipette and sterile pipette tips
6.8 Surface disinfecting solution (e.g., 10% bleach or 70% ethanol)
6.9 Class II biosafety cabinet

7.0. PROCEDURAL STEPS

7.1. Storing and Handling Disposables

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

b. 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 if needed by heating the base pack overnight in your incubator. To degas buffer:

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

3. 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.

4. 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.

c. 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.

7.2. Sample Preparation Procedures

a. Incubate and Inspect the Samples

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

    1. Using a pure isolated colony as the source, streak BHI agar plates heavily in the upper portion of the plate to create a lawn. Streak the remainder of the plate lightly to create single colonies.

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

b. Transfer Sample Buffer to Intermediate Tubes

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

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

c. 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.

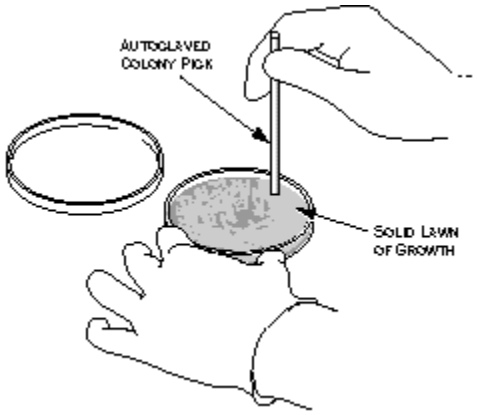
2. <u>For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube</u>.

   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.

d. Harvest the Samples

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



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

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

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

e. 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.*

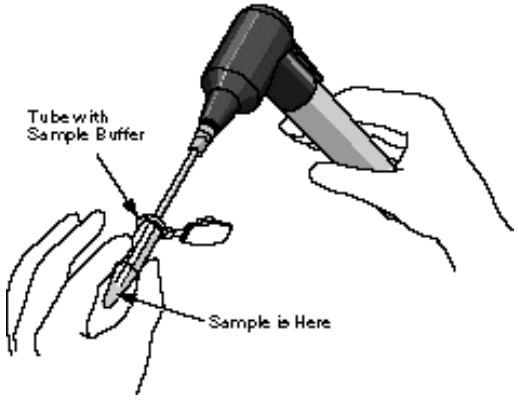
3. Press the ON lever on the mixer for about 5 seconds.

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

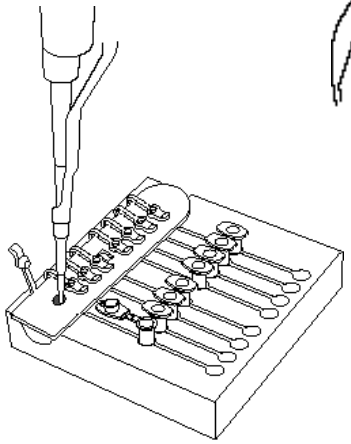
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.

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.



7.3. Transfer the Samples to the Sample Carrier



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

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

c. Close the lid cover for the well.

d. 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.*

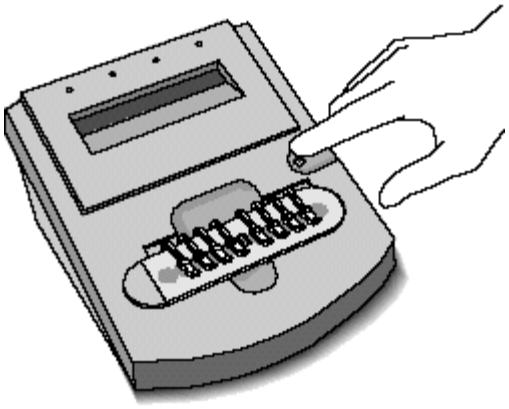
e. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).

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

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

a. 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**.



a. 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.

a. 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.*

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

a. 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.*

7.6. Creating and Loading a Batch

a. There are three options under the Operations menu for creating standard batches:
   - *EcoR*I batches (VCA)
   - *Pst*I batches (VCB)
   - *Pvu*II batches (VCC)

   You can also create special batches:
   - Restriction Enzyme Flexibility batches
   - **Substitute Enzyme batches (including *Hin*d III)**

b. From the Instrument Control Base Window:

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

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

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

samples. These become required fields and the system will make you enter data before you can save the information in this window.

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

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

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

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

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

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

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

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

10. Repeat for the other seven samples.

11. Click on Save and Submit Batch to Instrument.

7.7. Loading Disposables

a. 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 if 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.*

b. 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.*

c. Load the Sample Carrier

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

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

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

d. Load the DNA Prep Carrier

   1. Remove the DNA Prep carrier from the refrigerator.

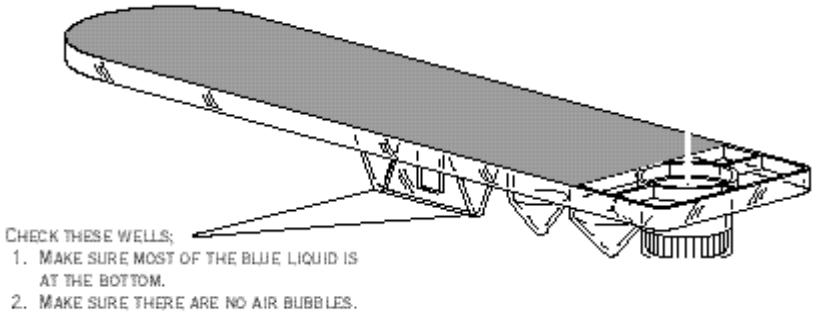
   2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**

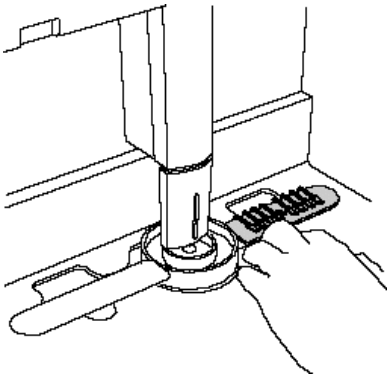
   3. **CAUTION!** Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.

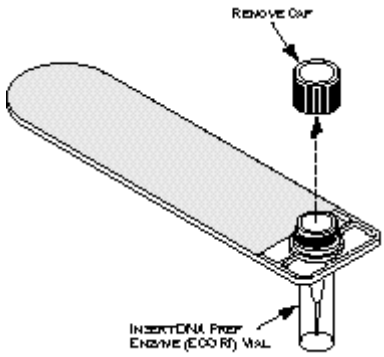
4. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as a 50 U/µL working stock as follows.**

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

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





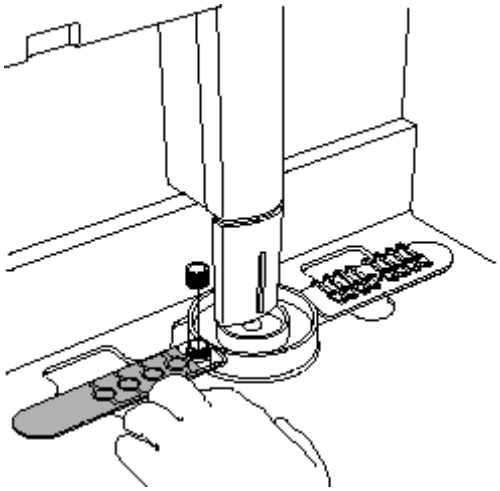


5. Remove the cap from the Enzyme vial.

6. Insert the vial into the carrier.

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

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

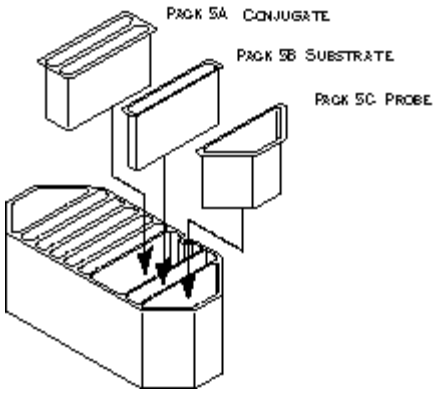


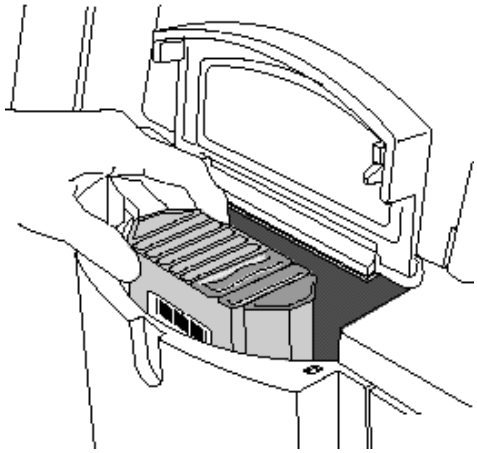
e. Load the MP Base and Carousel

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.



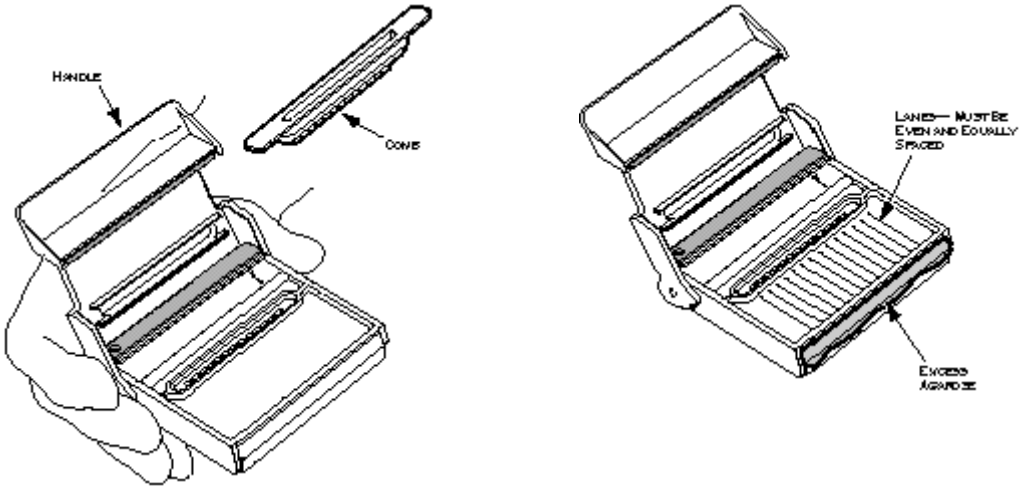


CAUTION! *Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You*

could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.

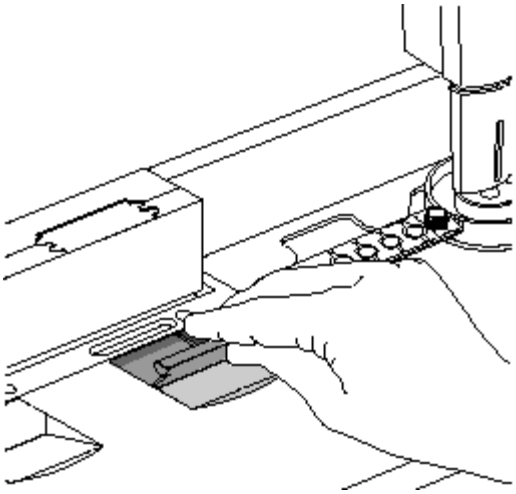
f. Load the Gel Cassette

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.

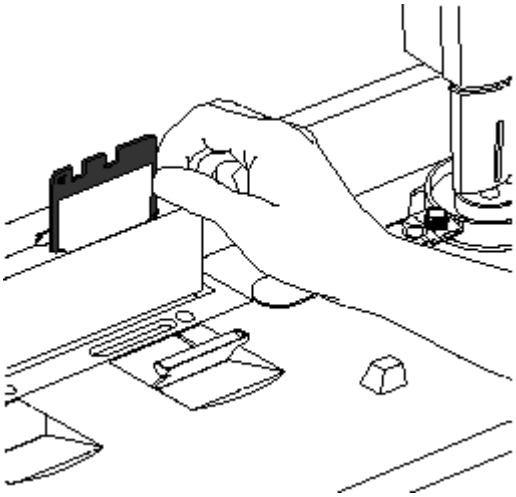


6. Press the cassette forward firmly until it snaps into place.

g. 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.

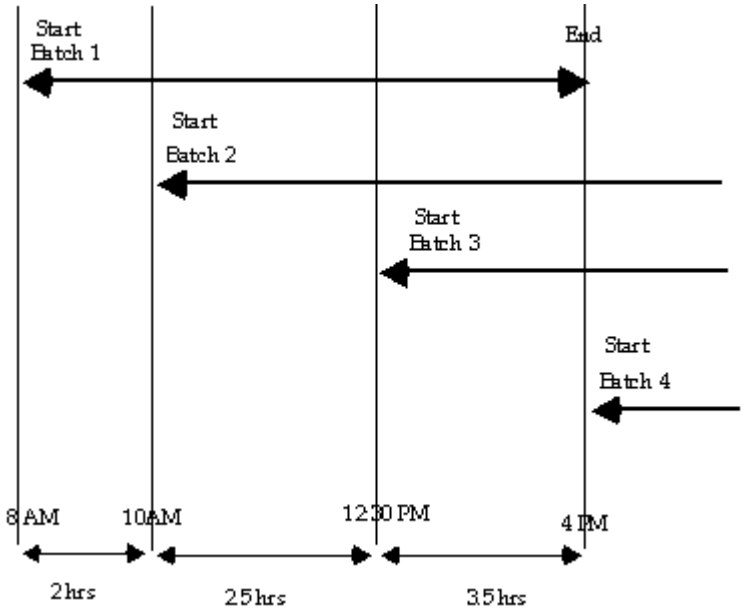
   CAUTION! *You can insert the membrane backwards. This will cause an alarm that prevents the sample from being processed until the error is corrected. Always make certain that the two large slots are on top and that the square hole on the side faces your left as you insert the membrane.*



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

i. 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 below shows the approximate loading times for each batch in a work shift using only the VCA protocol.



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

3. 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.

7.8. 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.

8.0. QUALITY ASSURANCE AND QUALITY CONTROL

A positive control (*E. coli* QC101) is processed each day the RiboPrinter is run.

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.

DuPont. 2013. DUPONT™ RIBOPRINTER® SYSTEM. DuPont, Wilmington, DE.

10.0. REVISION HISTORY

Revision	Date	Responsible Person	Description of Change
1	June 2015	Elizabeth Casarez	Initial Release
2	August 2018	Lucas Gregory	Update UTH Lab name
3	August 2022	Anna Gitter	Updated UTH Lab name