

TEXAS A&M AGRILIFE RESEARCH
TEXAS WATER RESOURCES INSTITUTE

Statewide Bacterial Source Tracking Program for FY 2016

FY16 State NPS Grant Program
TSSWCB Project No. 16-51
AgriLife Project No. 06-407693

Quarter No. 4 From: 9/01/2016 through 11/30/2016

I. Abstract

Water sample processing continued this quarter with six additional samples being processed and eight *E. coli* isolates per water sample being isolated. Planning for known source fecal sample collections in the Big Elm Creek and Plum Creek watersheds continued. Several unexpected equipment maintenance issues arose this quarter and were resolved or plans have been made to make the repairs next quarter. Education and outreach regarding BST approaches, application and utility were provided at multiple events. Discussions focused on pollutant source identification, quantitative microbial risk assessment and application of BST in groundwater wells. Plans are in place for a team meeting next quarter to discuss progress and for known source fecal sample collections to begin.

II. Overall Progress and Results by Task

Task 1 Project Administration

Subtask 1.1 TWRI will prepare electronic quarterly progress reports (QPRs) for submission to the TSSWCB. QPRs shall document all activities performed within a quarter and shall be submitted by the 15th of December, March, June and September. QPRs shall be distributed to all Project Partners.

The following actions have been completed during this reporting period:

- a. The 4th quarterly progress report was submitted on December 14, 2016.

50% Complete

Subtask 1.2 TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly.

The following actions have been completed during this reporting period:

- a. To date, \$185,825, or approximately 42% of the \$444,170.00 has been expended.

45% Complete

Subtask 1.3 TWRI will host coordination meetings or conference calls, at least quarterly, with Project Partners to discuss project activities, project schedule, communication needs, deliverables, and other

requirements. TWRI will develop lists of action items needed following each project coordination meeting and distribute to project personnel.

The following actions have been completed during this reporting period:

- a. A project team meeting was held in College Station on October 3rd to discuss project progress and ongoing activities.

50% Complete

Subtask 1.4 TWRI will work with AgriLife SCSC and UTSPH EP to develop a Final Report that summarizes activities completed, conclusions reached, and extent to which project goals and measures of success have been achieved.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Task 2 Quality Assurance

Subtask 2.1 TWRI will work with UTSPH EP and AgriLife SCSC to develop a QAPP for activities in Tasks 3-5 consistent with the most recent versions of EPA Requirements for Quality Assurance Project Plans (QA/R-5) and the TSSWCB Environmental Data Quality Management Plan.

The following actions have been completed during this reporting period:

- a. The QAPP was approved by TSSWCB on April 6, 2016.
- b. Task complete.

100% Complete

Subtask 2.2 TWRI, UTSPH EP and AgriLife SCSC will implement the approved QAPP and submit revisions and amendments as needed.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 2.3 AgriLife SCSC and UTSPH EP will maintain and update the 7 statewide BST template-SOPs for collection of fecal samples for BST, isolation of E. coli, archival of E. coli isolates, ERIC-PCR, RP, pre-processing of water samples for Bacteroidales PCR, and Bacteroidales PCR consistent with EPA Guidance for Preparing Standard Operating Procedures (SOPs) (QA/G-6) and the TSSWCB Environmental Data Quality Management Plan so that they include the most recent advances in BST science, methodologies, markers and technologies.

The following actions have been completed during this reporting period:

- a. SOP updates were discussed during the team meeting. Reviews are underway and any needed updates should be completed next quarter.

45% Complete

Task 3 BST Analyses

Subtask 3.1 UTSPH EP and AgriLife SCSC will maintain BST analytical equipment (e.g., RiboPrinter) and general laboratory equipment to support BST analyses. This includes securing maintenance contracts, replacement parts, and expendable supplies.

The following actions have been completed during this reporting period:

- a. UTSPH EP has a refrigerator and ultra-low temperature (-80°C) freezer that need repairs. Service visits have been scheduled.
- b. AgriLife SCSC's RiboPrinter camera malfunctioned and was replaced. There was no charge from DuPont for this since the defective camera had just been installed during the summer 2016 PM visit.
- c. The water feed line to AgriLife SCSC's DI water unit ruptured flooding areas of the lab and surrounding rooms. The line has been temporarily repaired, but plans are being discussed to install different tubing/pipes and additional valves to decrease the chance of future leaks.

50% Complete

Subtask 3.2 UTSPH EP and AgriLife SCSC will retain (or hire) lab personnel, students, and/or Postdoctoral Research Associates to maintain laboratory operating capacities and technical expertise to conduct BST studies across the state.

The following actions have been completed during this reporting period:

- a. UTSPH EP has retained Dr. Joy Truesdale and Dr. Elizabeth Casarez (part-time) to assist with project activities.
- b. AgriLife SCSC has retained Maitreyee Mukherjee as a postdoctoral research associate.

100% Complete

Subtask 3.3 UTSPH EP will perform BST analysis to support watershed planning efforts in the Big Elm Creek watershed. BST analyses will be performed on monthly samples from 2 sites (Segment 1213A). Sample collection will be conducted by TWRI with funding provided by TCEQ.

The following actions have been completed during this reporting period:

- a. Water samples were collected from 2 sites in the Big Elm Creek watershed each month of the quarter. Eight isolates from each sample were isolated, confirmed as *E. coli* (modified mTEC and NA-MUG positive) with a total of 48 isolates archived. ERIC-PCR has been completed for approximately 80% of water isolates collected to date, while RiboPrinting has been completed for approximately 90%.

80% Complete

Subtask 3.4 AgriLife SCSC will perform BST analysis to support watershed protection plan implementation in the Plum Creek watershed. BST analyses will be performed on monthly samples from 5 sites [sampling plan to be determined following discussions with GBRA and Plum Creek stakeholders] in the Plum Creek watershed. Samples will be collected by GBRA with funding from TSSWCB.

The following actions have been completed during this reporting period:

- a. AgriLife SCSC began receiving monthly sampling plates from GBRA in September. *E. coli* have been isolated from the plates and archived for BST.

10% Complete

Task 4 Known Source Fecal Sample Collection

Subtask 4.1 TWRI will work with UTSPH EP and AgriLife SCSC to develop a targeted list of needed species for fecal sample collection and plan for their collection and delivery. This list should primarily fill gaps in the Texas E. coli BST Library and provide support for analyses in the Big Elm Creek and Plum Creek watersheds.

The following actions have been completed during this reporting period:

- a. Known source sampling targets were established this quarter and are as follows:
Human WWTF (8); Human OSSF (8); Cattle (6); Horses (2); Sheep (1); Goats (1); Chickens (2); Turkeys (2); Feral Hogs (10); various small and medium mammals (10)
- b. Task complete.

100% Complete

Subtask 4.2 TWRI will collect 50 fecal samples from each watershed in accordance with the plan developed in Subtask 4.1 and work closely with UTSPH EP and AgriLife SCSC to coordinate delivery of the samples to the appropriate lab. TWRI will communicate with a select group of organizations, agencies and businesses in each of the 2 targeted watersheds to arrange and resolve any access concerns and gather input to improve geographic targeting of sample collection. Travel plans, scheduling, and routing maps will be prepared prior to deploying the field crew. TWRI will deploy the field crew to collect known source samples from each targeted watershed. TWRI will coordinate closely with UTSPH EP and AgriLife SCSC to ensure sample delivery adheres to established QA/QC procedures. A known source sample data set will be finalized after completion of the field work and submitted to TSSWCB.

The following actions have been completed during this reporting period:

- a. TWRI has met with and secured access to one property in the Big Elm Creek watershed and has visited with several others.
- b. Sample collection will begin next quarter after deer season and when equipment issues in the lab have been resolved.
- c. Efforts will continue next quarter to secure access for sample collections.

25% Complete

Task 5 BST Library Refinement and Library Independent Marker Development

Subtask 5.1 UTSPH EP and AgriLife SCSC will isolate E. coli from the approximately 100 known source fecal samples collected through Task 4. Approximately three isolates from each fecal sample (for a total of approximately 300 isolates) will be analyzed using ERIC-PCR for inclusion in the Texas E. coli BST Library; based on the ERIC-PCR fingerprint patterns, approximately half of the isolates (150) will be further analyzed using RP for inclusion in the Texas E. coli BST Library. UTSPH EP and AgriLife SCSC will equitably split workload.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 5.2 UTSPH EP and AgriLife SCSC will collaborate to evaluate the geographical and temporal stability, composition, average rates of correct classification (accuracy), diversity of source specific isolates, and further development and refinement needs of the Texas E. coli BST library, as the library is updated with new known-source isolates.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 5.3 As funding allows, AgriLife SCSC and UTSPH EP will utilize the best available bacterial indicators to evaluate and further develop/refine source-specific bacterial PCR markers using known source fecal material. AgriLife SCSC and UTSPH EP efforts will focus on evaluating additional library-independent PCR markers for the Texas BST toolbox. UTSPH EP will also archive Big Elm Creek water DNA samples for future library independent work.

The following actions have been completed during this reporting period:

- a. UTSPH EP discussed selection and field application of real-time qPCR library independent markers for human, bird and dog fecal pollution with collaborators Dr. Jody Harwood (University of South Florida) and Joe Hernandez (Scottsdale Water, Arizona).

10% Complete

Task 6 Outreach

Subtask 6.1 TWRI will host and maintain the <http://texasbst.tamu.edu> website to disseminate educational materials, project updates, science updates, notify readers about educational opportunities, and other outreach efforts to advance the science and application of BST in Texas and nationally.

The following actions have been completed during this reporting period:

- a. TWRI continues to host and maintain the Texas BST Library website. Between 9/1/2016 – 11/30/2016, there were 46 visits to the website by 39 unique visitors.
- b. A website revision is currently underway to update the website with more timely information.

50% Complete

Subtask 6.2 TWRI, UTSPH EP, and AgriLife SCSC will promote the use of and provide resources on BST. TWRI, UTSPH EP, and AgriLife SCSC will distribute educational brochures developed. As needed, TWRI, UTSPH EP, and AgriLife SCSC will develop additional flyers, one-pagers, tri-folds or other appropriate printed media, that can be used to 1) discuss the appropriate application of BST in identifying fecal contamination sources and 2) promote the analytical laboratory capability of public BST labs which the State has invested. As appropriate, TWRI will include information about BST in general, and this project

specifically, in the txH2O magazine and Conservation Matters e-mail newsletter. Finally, TWRI, UTSPH EP, and AgriLife SCSC will periodically meet with natural resource agencies, public and private laboratories, and other researchers/academia to advance the general knowledge and understanding of BST and appropriate methodologies and SOPs for use of BST in Texas.

The following actions have been completed during this reporting period:

- a. BST application information was presented at the Watershed Coordinator Steering Committee meeting in Columbus on September 9th.
- b. AgriLife SCSC gave presentation on “Bacterial Source Tracking” to the TAMU Student Chapter of the Soil and Water Conservation Society, 17 October 2016.
- c. UTSPH EP provided additional BST information to TIAER for discussions with Tarrant Regional Water District regarding potential BST work in their service area.
- d. Following the discussion on BST with the Galveston Bay Estuary Program, a preproposal was submitted to conduct BST project on Galveston Bay tributaries, a full proposal was requested, and word has arrived that they intend to fund the work.

50% Complete

III. Projected Work for Next Quarter

- Continue processing water samples for BST analysis. The last set of Big Elm Creek water samples will be collected in January 2017
- Continue ERIC-PCR and RiboPrinting *E. coli* isolates from processed water samples
- Begin collecting known source fecal samples
- Plan additional known source fecal sample collection events
- Process known source samples for BST analysis; complete ERIC-PCR and RiboPrinting analyses as samples are received and processed
- Host project conference call