

TEXAS WATER RESOURCES INSTITUTE

Statewide Bacterial Source Tracking Program for FYs 2013-2014
FY 2013 Workplan 13-50

Quarter no. 7 From 6/1/14 Through 8/31/14

I. Abstract

Revision 1 of the QAPP was approved on August 14. AgriLife SCSC met with UTSPHEP in El Paso on August 12 to discuss BST projects and protocols and tour UTSPHEP lab and facilities. At this meeting, UTSPHEP and AgriLife SCSC discussed plans for updating SOPs - specifically the decloning protocols for selecting isolates to be RiboPrinted based on ERIC patterns. UTSPHEP used the Texas *E. coli* BST Library (ver. 6-13) to identify 50 water *E. coli* ERIC-RP fingerprints for AgriLife SCSC to assist in their project with the San Antonio River Authority. Also, UTSPHEP received 12 water samples from Houston and processed the water for *Bacteroidales*, IDEXX counts, mTEC counts, and BST archiving. Work continued to expand the statewide *E. coli* BST library this quarter. AgriLife SCSC has completed fingerprinting on 'A' and 'C' isolates and as well as RiboPrinting the additional 48 *E. coli* cultures ('B' isolates) that were sent to AgriLife SCSC by UTSPHEP. AgriLife SCSC has requested and received 38 additional isolates from UTSPHEP and is currently in the process of conducting RB analysis on them. Further, UTSPHEP completed ERIC-PCR for 144 isolates. AgriLife SCSC has completed evaluation of the specificity and sensitivity of the Brevibacterium (LA35) poultry marker. TWRI presented a "Review of Bacterial Source Tracking in Texas" to numerous natural resource agencies and organizations on July 31 at the Texas Watershed Roundtable. Additionally, presentations were made on Microbial Source Tracking at the *HGAC CWI Workshop* on July 17 in Houston and *Soil in the City Conference* on 29 June - 2 July in Chicago, IL.

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 March, June, September and December. QPRs shall be distributed to all project partners and posted on the project website.

The following actions have been completed during this reporting period:

- a. The 7th quarterly progress report was submitted on September 15, 2014.

72% 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, \$339,358 of the \$454,098 has been expended.

75% Complete

Subtask 1.3 TWRI will host coordination meetings or conference calls with the TSSWCB, UTSPHEP and AgriLife SCSC, at least quarterly, 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. On July 8, 2014, a BST coordination meeting was held to discuss project activities and schedule.

72% Complete

Subtask 1.4 TWRI will work with AgriLife SCSC and UTSPHEP to develop a Final Report that summarizes activities completed, conclusions reached during the project, and the 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 UTSPHEP, AgriLife SCSC and IRNR to develop a QAPP for activities in Tasks 3 and 4 EPA Requirements for Quality Assurance Project Plans (QA/R-5) (May 2006) and the TSSWCB Environmental Data Quality Management Plan (August 2007).

The following actions have been completed during this reporting period:

- a. The QAPP was approved by the TSSWCB on August 5, 2013.

100% Complete

Subtask 2.2 TWRI will submit revisions and necessary amendments to the QAPPs as needed.

The following actions have been completed during this reporting period:

- a. Revision 1 of the QAPP was approved on August 14, 2014.

100% Complete

Subtask 2.3 AgriLife SCSC and UTSPHEP will maintain and update, at least annually, 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. At their August 2014 meeting, UTSPHEP and AgriLife SCSC discussed plans for updating SOPs in the coming quarters. Specifically, UTSPHEP and AgriLife SCSC discussed the decloning protocols in selecting the isolates to be RiboPrinted based on ERIC patterns. It was discussed how best to handle the scenario of when 1 ERIC pattern is within the 80% similarity cutoff to

each of the other two isolates from that sample, but that those two isolates would not be considered similar to each other. To maximize the diversity of the library, it was decided that for that scenario to select the two extreme patterns vs the one middle pattern.

20% Complete

Subtask 2.4 AgriLife SCSC and UTSPHEP will coordinate to ensure that needed personnel training is kept on par between the groups to ensure congruity statewide.

The following actions have been completed during this reporting period:

- a. AgriLife SCSC personnel (Gentry and Wanjugi) met with UTSPHEP (Di Giovanni, Casarez, Truesdale) in El Paso on August 12 to discuss BST projects and protocols and tour UTSPHEP lab and facilities.

72% Complete

Subtask 2.5 UTSPHEP and AgriLife SCSC will work with public and private laboratories across the state which are exploring the use of BST. UTSPHEP and AgriLife SCSC will work to ensure that methodologies and QA/QC mechanisms adopted by these other laboratories are as congruent as possible with SOPs utilized by UTSPHEP and AgriLife SCSC (subtask 2.1)

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

72% Complete

Task 3 Known Source Fecal Sample Collection

Subtask 3.1 TWRI will work with IRNR to collect known source fecal samples.

The following actions have been completed during this reporting period:

- a. IRNR collected 100 known source fecal samples (75 from the San Antonio and 25 from the Leon) and shipped them to UTSPHEP for isolation and analysis. See attached list of fecal samples collected.

100% Complete

Subtask 3.2 TWRI and IRNR will work with UTSPHEP and AgriLife SCSC to develop a targeted list of needed species/watersheds for fecal sample collection and plan for their collection and delivery. This list should primarily fill gaps in the Texas E. coli BST Library identified in other TSSWCB-funded BST projects. Targeted species will include small mammals such as mice, squirrels, nutria and rabbits. In addition, samples will be collected from at least one previously studied watershed (e.g., Leon River) in order to determine the temporal stability of the Texas E. coli BST Library. Approximately 50 known source fecal samples from each of 2 watersheds (Leon and San Antonio Rivers) are budgeted for collection (total of 100 samples).

The following actions have been completed during this reporting period:

- a. A targeted list of needed species/watersheds for fecal sample collection was developed and provided to IRNR to guide collection (see QPR3 for the list).

100% Complete

Subtask 3.3 IRNR will collect fecal samples in accordance with the plan developed in Subtask 3.2 and work closely with UTSPHEP and AgriLife SCSC to coordinate delivery of the samples to the appropriate lab. IRNR 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. IRNR will deploy the field crew to collect known source samples from each targeted watershed. IRNR will coordinate closely with UTSPHEP 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 TWRI.

The following actions have been completed during this reporting period:

- a. IRNR collected 100 known source fecal samples (75 from the San Antonio and 25 from the Leon) and shipped them to UTSPHEP for isolation and analysis. See attached list of fecal samples collected.

100% Complete

Task 4 Analytical Laboratory Capacity, Library Expansion and Methods Development

Subtask 4.1 UTSPHEP and AgriLife SCSC will maintain BST analytical equipment (e.g., RiboPrinter) and general laboratory equipment. This includes securing maintenance contracts, replacement parts and expendable supplies and purchase of a new computer for the UTSPHEP RiboPrinter system.

The following actions have been completed during this reporting period:

- a. UTSPHEP and AgriLife SCSC continuously maintain BST analytical equipment and general laboratory equipment.
- b. The UTSPHEP RiboPrinter required two replacement parts (ST units). They have been installed and are performing within specifications.

90% Complete

Subtask 4.2 UTSPHEP will retain (or hire) a Graduate Student or Postdoctoral Research Associate that will 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) aid in the evaluation, expansion and maintenance of the Texas E. coli BST Library, 3) evaluate library-independent methods and markers, and 4) provide support of TSSWCB project 12-10 BST to Support Adaptive Management of the Arroyo Colorado WPP.

The following actions have been completed during this reporting period:

- a. UTSPHEP has retained Joy Truesdale and Elizabeth Casarez and hired one graduate student, Cesar Navar, part-time to assist with project activities.
- b. UTSPHEP used the Texas E. coli BST Library (ver. 6-13) to identify an additional set of 50 water E. coli ERIC-RP fingerprints for AgriLife SCSC to assist in their project with the San Antonio River Authority.
- c. UTSPHEP received 12 water samples from Houston and processed the water for Bacteroidales, IDEXX counts, mTEC counts, and BST archiving.

100% Complete

Subtask 4.3 AgriLife SCSC will retain (or hire) Graduate Students and/or a Postdoctoral Research Associate that will 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) continue BST efforts in support of TSSWCB projects 09-10 Development of a WPP for Attoyac Bayou, 11-50 Assessment of Water Quality and Watershed Planning for the Leona River and 11-51 Instream Bacteria Influences from Bird and Bat Habitation of Bridges, and 3) evaluate new poultry marker(s) for library-independent BST.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter

100% Complete

Subtask 4.4 UTSPHEP and AgriLife SCSC will expand the statewide E. coli BST library through the analysis of ERIC-RP data provided by AgriLife SCSC for approximately 100 E. coli known source isolates obtained from the Leona River watershed (TSSWCB Project 11-50). Additionally, UTSPHEP and AgriLife SCSC will isolate E. coli from approximately 100 known source fecal samples collected through Task 3, which should primarily fill gaps in the library identified in other TSSWCB-funded BST projects. Approximately three isolates from each fecal sample (for a total of approx. 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. UTSPHEP and AgriLife SCSC will equitably split workload. AgriLife SCSC will also fingerprint (ERIC-RP) and analyze 20 known-source E. coli isolates collected as part of TSSWCB Project 11-51.

The following actions have been completed during this reporting period:

- a. AgriLife SCSC has completed fingerprinting on 'A' and 'C' isolates and as well as RiboPrinting the additional 48 E. coli cultures ('B' isolates) that were sent to AgriLife SCSC by UTSPHEP.
- b. Following fingerprinting and RiboPrinting of the isolates mentioned above, AgriLife SCSC identified 38 additional E. coli isolates that needed further RB analysis. AgriLife SCSC has requested and received these isolates from UTSPHEP and is currently in the process of conducting RB analysis on them.
- c. UTSPHEP has completed ERIC-PCR for 144 isolates. However, 37 isolates require redos to give ~75% completion rate. 71 isolates thus far have been selected for RiboPrinting with RiboPrinting completed for 56 isolates (~79%). UTSPHEP assisted AgriLife SCSC in verifying the list of 38 additional isolates selected for RiboPrinting (see discussion section 2.3) that need to be sent to AgriLife SCSC (slants sent 9-10-14).

80% Complete

Subtask 4.5 UTSPHEP 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.

The following actions have been completed during this reporting period:

- a. UTSPHEP began Initial characterization of non-host specific (cosmopolitan) E. coli strains.

50% Complete

Subtask 4.6 Using known source fecal material, AgriLife SCSC and UTSPHEP will utilize best available bacterial indicators to evaluate and further develop/refine source-specific bacterial PCR markers. Efforts

will be made on markers to 1) identify poultry litter/manure pollution, 2) evaluate using genetic targets based on ERIC-PCR products to differentiate human and animal E. coli, 3) differentiate between domestic and feral hogs, 4) differentiate deer from other ruminants by analysis of existing data on deer fecal microbial communities, and 5) evaluate occurrence of HF183 cross reactivity for known source samples.

The following actions have been completed during this reporting period:

- a. AgriLife SCSC has completed evaluation of the specificity and sensitivity of the *Brevibacterium* (LA35) poultry marker using 58 specific (fecal/litter samples from poultry) and 121 non-specific fecal samples (fecal samples from other non-target species).
 - a. Overall, the LA35 marker was sensitive to 46 out of the 58 specific samples (79%) and specific to 119 out of the 121 non-samples (98%) tested. The two non-specific samples that tested positive for the LA35 marker were: A Black Angus Heifer (Leona); and A Duck (Attoyac).
- b. UTSPHEP and AgriLife SCSC exchanged hog and avian known source fecal samples to aid in testing marker development.
- c. UTSPHEP tested a total of 101 known source fecal samples from Task 3 for the *Bacteroidales* GenBac (Bac32F) and Human (HF183) PCR markers.
 - a. 11 samples tested negative (two attempts) for the GenBac marker and included 7/16 raccoon, 2/7 skunk, 1/12 opossum, and 1/13 deer mouse. This may have been due to PCR inhibition. Dilutions of fecal DNA will be analyzed in an attempt to overcome possible PCR inhibition.
 - b. 10 non-human samples tested positive (two attempts) for the Human HF183 marker and included 3/13 domestic goat, 2/12 opossum, 2/16 feral hog, 1/1 owl, 1/13 deer mouse, and 1/16 raccoon.
 - c. 16/16 feral hog samples were positive for the hog marker.

50% Complete

Subtask 4.7 AgriLife SCSC and UTSPHEP will cooperate with other entities nationwide to ensure that the most up-to-date and accurate BST approaches are implemented in Texas by attending and participating in BST-related meetings, seminars and workshops, as appropriate, to learn of new and improved BST methods being employed elsewhere.

The following actions have been completed during this reporting period:

- a. UTSPHEP has been collaborating with a group of BST investigators across the US to address the possibility of deer fecal contamination resulting in HF183 *Bacteroidales* human marker false positive results.
- b. Dr. Norm Neumann, University of Alberta-Edmonton, discussed opportunities to collaborate with UTSPHEP on the development of a novel *E. coli* library independent BST method.

72% Complete

Task 5 Outreach on Bacterial Source Tracking and BMPs

Subtask 5.1 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 6/1/14 – 8/31/14, there were 40 visits to the website by 35 unique visitors. Since project inception in October 1, 2012, there have been 751 visits to the website by 540 unique visitors.

72% Complete

Subtask 5.2 TWRI, UTSPHEP, and AgriLife SCSC will periodically meet with natural resource agencies, including but not limited to USEPA-R6, TCEQ, TPWD, TDA, GLO, DSHS, and selected river authorities, to advance the general knowledge and understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the use of BST in Texas.

The following actions have been completed during this reporting period:

- a. TWRI presented a “Review of Bacterial Source Tracking in Texas” on July 31, 2014 at the Texas Watershed Roundtable in Waco. Staff from TTU, EPA, AgriLife Research and Extension, HGAC, TPWD, TIAER, NRCS, Nueces River Authority, TCEQ, TSSWCB, Texas A&M Forest Service, Tarrant Regional Water District, Guadalupe-Blanco River Authority, North Central Texas Council of Governments, and other organizations were present. .

72% Complete

Subtask 5.3 TWRI, UTSPHEP, and AgriLife SCSC will distribute the educational brochures developed through TSSWCB Project 10-50 (subtask 4.2). TWRI, UTSPHEP, and AgriLife SCSC will develop additional flyers, one-pagers, tri-folds or other appropriate printed media, as needed, 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, the Conservation Matters e-mail newsletter and AgriLife Today news.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

72% Complete

Subtask 5.4 TWRI, UTSPHEP, and AgriLife SCSC will promote the use of and provide resources on BST by participating in conferences, workshops, seminars and other appropriate venues, including but not limited to the 2013 and 2014 TCEQ Environmental Trade Fair, WEF/WEAT events in Texas, TSCRA/TFB/TWA annual conventions and ASABE events in Texas.

The following actions have been completed during this reporting period:

- a. SCSC presented on “Use of bacterial source tracking to characterize fecal source contributions in watersheds” at the Soil in the City Conference on 29 June - 2 July 2014 in Chicago, IL.
- b. TWRI and SCSC presented on Microbial Source Tracking at the HGAC CWI Workshop on July 17, 2014 in Houston.
- c. TWRI presented a “Review of Bacterial Source Tracking in Texas” on July 31, 2014 at the Texas Watershed Roundtable in Waco.

72% Complete

Subtask 5.5 TWRI, UTSPHEP and AgriLife SCSC will work to inform other researchers/academia who are engaged in BST in Texas (e.g., Edrington, Brinkmeyer, Alam, Ward) about the methods and approaches recommended by the Task Force and being implemented by the State.

The following actions have been completed during this reporting period:

- a. SCSC and TWRI discussed status of BST with Dr. Brinkmeyer during HGAC CWI Workshop on July 17, 2014 in Houston.

72% Complete

Subtask 5.6 To build on the success of the 2012 BST – State of the Science Conference, TWRI, UTSPHEP, and SCSC will evaluate the need for and timing of a follow-up conference. If the need is substantiated, TWRI, UTSPHEP and SCSC will initiate planning and logistics for a follow-up conference.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 5.7 With assistance from the USDA-NASS Texas Field Office, a stratified random sampling scheme will be implemented using a target population of beef cattle producers who completed 2012 Census of Agriculture forms. The sample will be stratified according to NASS district and beef cattle herd size. USDA-NASS will provide Texas A&M Department of Soil & Crop Sciences with a list of unique identifying numbers that will be placed on all survey materials so that response/non-response can be tracked. The USDA-NASS Texas Field Office will also assist with logistics related to compiling, stuffing, and mailing survey materials that will include an introductory postcard, the first survey packet with cover letter and survey instrument, a reminder postcard, and a second survey packet with cover letter and survey instrument. This information will support assessment of barriers to BMP adoption in conjunction with TSSWCB Project #12-08.

The following actions have been completed during this reporting period:

- a. Data collection was ceased on November 1, 2013. Twenty postcards and/or survey packets were returned undeliverable, 16 individuals reported they had sold all of their cattle, and 46 individuals indicated they did not wish to participate in the study. This yielded a frame error of 4.8% and reduced the total sample to 1,618 beef cattle producers. A total of 90 surveys (5.6%) were completed online and 687 (42.5%) were completed on paper and mailed back to the student researcher for a total response rate of 48.1%. The completed surveys are currently being scanned by the Department of Agricultural Leadership, Education, and Communications at Texas A&M University. It is anticipated the data file will be ready for analysis by the second week of December. Data analysis and reporting of the results will continue through Spring 2014.

100% Complete

Subtask 5.8 In order to reduce pollutant contributions to streams, including bacteria, TWRI will coordinate a Southwestern United States Stream Restoration Conference Workshop titled: Riparian Vegetation Workshop – Putting the ‘green’ into streambank stabilization in San Antonio in 2013.

The following actions have been completed during this reporting period:

- a. The Southwest Stream Restoration Conference was held on May 28-30 in San Antonio. The conference was attended by more than 230 participants and the Riparian Vegetation Workshop, held on May 28 from 1:30-5:00 pm had more than 50 attendees. The conference agenda and presentations are available at http://southweststream.org/?page_id=21.
- b. TWRI chaired the program planning committee for the 2014 Southwestern Stream Restoration Conference. The Conference was held on May 28-30, 2014 in San Antonio.

100% Complete

Task 6 Technical Assistance for Leon River WPP Update

Subtask 6.1 Schedule and attend a meeting to gain input and support from the stakeholders on the strategies, proposed answers and rebuttals of the comments submitted by EPA. Also participate on planning conference calls as needed.

The following actions have been completed during this reporting period:

- a. Parsons assisted with conducting a meeting of the Leon WPP Steering Committee on December 18, 2013 to garner input from the broader committee prior to finalizing the response and submitting them to EPA.
- b. Parsons worked with TSSWCB and TWRI to prepare a final draft of the response to comments for distribution to the Leon WPP Steering Committee in March. This document will be used to guide revisions to the WPP.

100% Complete

Subtask 6.2 PowerPoint presentation to stakeholders to summarize key issues of response to comments for discussion at stakeholder meeting.

The following actions have been completed during this reporting period:

- a. Parsons prepared a PowerPoint presentation for the December 18 Leon WPP Steering Committee meeting summarizing key issues of response to comments for discussion at the meeting.

100% Complete

III. Related Issues/Current Problems and Favorable or Unusual Developments

- The Bacterial Source Tracking Team received the 2014 Texas A&M University College of Agriculture and Life Sciences Dean's Outstanding Achievement Award for Interdisciplinary Research.

IV. Projected Work for Next Quarter

- SCSC will complete evaluation of poultry marker
- SCSC and UTSPHEP will complete revision of BST SOPs
- UTSPHEP will complete the ERIC and RP processing for their Infrastructure isolates, get patterns from AgriLife SCSC, and begin library analysis.