Texas Water Resources Institute

Support Analytical Infrastructure and Further Development of a Statewide Bacterial Source Tracking Library
FY 10 State General Revenue Nonpoint Source Grant Program
TSSWCB Project No. 10-50

Quarter no. 7 from 03/01/12through 05/31/12

I. Abstract

Work this quarter has focused on the continuation of many project tasks. Following the hosting of the Statewide BST conference last quarter, numerous thoughts and ideas on how best to proceed with the project have arisen. Many activities are nearing completion and reports for these activities are being drafted. Texas BST Library additions have been made this quarter as well as progress on quantifying *E. coli* production from various known source samples. Collectively, the project is nearing completion and it has been realized that several tasks within the project will likely not be completed within this project timeline. Plans of action for these tasks have been established with TSSWCB to close these tasks out appropriately.

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. TWRI submitted the 7th Quarter, Year 2 report to TSSWCB on June 14, 2012.

87% 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. Expenditures and encumbrances thus far have totaled $288,516 or roughly 66% of total project funding.

70% Complete

Subtask 1.3: *TWRI will host coordination meetings, conference calls, or TTVN meetings with the TSSWCB, UTSPH-EPRC, 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. A project team meeting was held via conference call on May 17th to discuss a path forward for completing the project. All project collaborators were involved in this discussion.

87% Complete

Subtask 1.4: *TWRI will develop (Months 1-3), host and maintain (Months 4-24) a website (e.g., http://bst.tamu.edu) that will be used as a means 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. This quarter, the website http://texasbst.tamu.edu/ was viewed by 404 unique visitors with only 48% of them being new visitors to the site.

87% Complete

Subtask 1.5: *TWRI will work with AgriLife SCSC and UTSPH-EPRC to prepare Technical Reports as required by project Tasks into published technical reports. These reports will be permanently housed in the TWRI online Reports Database.*

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

TASK 2: Support and maintain BST analytical infrastructure

Subtask 2.1: *UTSPH-EPRC and AgriLife SCSC will ensure needed operational and maintenance support for current BST analytical equipment (i.e., RiboPrinter) and general laboratory equipment is executed. This includes securing maintenance contracts, replacement parts and expendable supplies.*

The following actions have been completed during this reporting period:

a. The RiboPrinter has begun jamming and will not complete automated processing. Steps are being taken to get DuPont back out to repair the machine.

87% Complete

Subtask 2.2: *UTSPH-EPRC will hire a Postdoctoral Research Associate/Research Associate that will maintain laboratory operating capacities and technical expertise to conduct BST studies across the state. This FTE is intended to provide support on TSSWCB-funded projects in the Leon River, Lampasas River, and Buck Creek watersheds (projects 10-51, 06-11, 06-07).*

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

100% Complete

Subtask 2.3: *AgriLife SCSC will hire a Postdoctoral Research Associate that will maintain laboratory operating capacities and technical expertise to conduct BST studies across the state. This FTE is intended to provide support on TSSWCB-funded projects in the Little Brazos River tributaries, Big Cypress Creek, and Attoyac Bayou watersheds (projects 09-52, 09-55, 09-10).*

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

100% Complete

TASK 3: Quality Assurance

Subtask 3.1: *AgriLife SCSC and UTSPH-EPRC will maintain and update (at least annually) statewide BST SOPs for ERIC-PCR, RP 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. No procedural changes have been made in ERIC RP or Bacteriodales PCR methods since the latest draft of SOPs were developed. As a result, no changes have been made to existing SOPs.
b. TSSWCB review of SOPs is still pending.

100% Complete

Subtask 3.2: *AgriLife SCSC and UTSPH-EPRC 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 and UTSPH-EPRC staff maintain regular communication to ensure consistency between

63% Complete

Subtask 3.3: *AgriLife SCSC and UTSPH-EPRC will work with AgriLife BAEN, USDA-ARS, USDA-NRCS WRAT, and others to develop strategies for 1) reconciling BST and modeling results (SELECT, SWAT, etc.) and 2) using BST in model development, calibration, and validation.*

The following actions have been completed during this reporting period:

a. Discussions have been held with selected experts regarding reconciling BST results with modeling outputs.
b. A meeting with additional experts is being planned for next quarter.

35% Complete

Subtask 3.4: *UTSPH-EPRC and AgriLife SCSC will work with public and private laboratories across the state which are exploring the use of BST. AgriLife EP 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 AgriLife EP and AgriLife SCSC (subtask 3.1).*

The following actions have been completed during this reporting period:

a. Conversations with multiple entities have been held regarding the general concepts and utility of BST. Entities typically expressed interest in conducting BST on their own and were seeking information. Follow up conversations have not materialized.
b. Attempts are being made to plan a conference call with some of these parties before the end of the project.

25% Complete

Subtask 3.5: *UTSPH-EPRC and AgriLife SCSC will work with AgriLife BAEN, USDA-ARS, USDA-NRCS WRAT, USGS, TIAER, and selected river authorities to define appropriate ambient water sampling protocols to provide desired statistical confidence with BST findings. The level of sampling adequate for statistical characterization of sources and determination of environmental conditions influencing source contributions (often wet versus dry weather conditions) will be described.*

The following actions have been completed during this reporting period:

a. Work on this task has focused on planning sampling regimes for specific projects. In these discussions, appropriate sampling regimes have been proposed; however, available budgets dictate what level of sampling is ultimately done.

b. In these discussions, more pertinent and realistic questions have been developed and include:

- Is collecting and processing duplicate water samples worth the cost?
- Within a realistic sampling regime, what samples are critical to collect?
- What is the benefit of collecting wet vs. dry weather samples?

25% Complete

TASK 4: Promotion of BST

Subtask 4.1: *AgriLife SCSC with assistance from TWRI will develop a publication that describes the extent of BST work conducted to date in Texas. This report should compare and contrast methodologies and results. This publication should build on the discussion in the Task Force Report.*

The following actions have been completed during this reporting period:

a. Relevant publications, presentations, and technical reports were collected from various sources. Started review of the materials.

20% Complete

Subtask 4.2: *TWRI will develop flyers, one-pagers, tri-folds or other appropriate printed media that can be used to 1) promote the general use of BST consistent with the Task Force Report, 2) discuss the appropriate application of BST in identifying fecal contamination sources, and 3) promote the analytical lab capability of public BST labs which the state has invested. Printed media should be appropriately developed for each target audience including, but not limited to, 1) state and federal agencies with water quality responsibilities and jurisdictions (TCEQ, TPWD, DSHS, GLO, USEPA (Region 6 and GOMP), USGS, NOAA), 2) political subdivisions of state government (municipal, county), including TML and TAC, that may be involved in watershed planning processes focused on abatement of bacterial contamination, 3) livestock producer organizations such as TFB, TSCRA, ICA, TAD, TCFA, TSGRA, TPPA, TPF, TWA, 4) private and public water quality labs, and 5) the general public. As appropriate, TWRI will include information about BST in general, and this project specifically, in the tx H2O, New Waves e-letter, AgriLife News.*

The following actions have been completed during this reporting period:

a. Informational handouts were printed and distributed at the BST Conference in February.

b. Work is underway to develop a mailing list of agencies, political subdivisions, special interest groups, laboratories and the general public. A total of 400 flyers will be mailed next quarter.

87% Complete

Subtask 4.3: *TWRI, UTSPH-EPRC, and AgriLife SCSC will promote BST by making presentations at conferences, workshops, seminars and other appropriate venues such as WEF/WEAT, TSCRA/TFB/TWA Annual Conventions, ASABE, TCEQ Environmental Trade Fair.*

The following actions have been completed during this reporting period:

a. Workshop on Bacterial Detection and Tracking was presented at the National Water Conference in Portland, OR, 22 May 2012. Kevin Wagner & Terry Gentry co-chaired the workshop and also gave BST presentations along with Emily Martin.
b. Emily Martin submitted abstract for presentation on "Bacterial Source Tracking (BST) in Action: Utilization of BST Toolbox Approach in Texas Watersheds" at the 20th Annual Nonpoint Source Monitoring Workshop in Tulsa, OK, 14-17 October 2012.

87% Complete

Subtask 4.4: *TWRI, UTSPH-EPRC and AgriLife SCSC will work to inform other researchers/academia who are engaged in BST in Texas (Edrington, Brinkmeyer, Alam) 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. No new activity to report.

80% Complete

TASK 5: Texas *E. coli* BST Library expansion, PCR marker development/refinement and *E. coli* isolate selection method evaluation

Subtask 5.1: *UTSPH-EPRC and AgriLife SCSC will isolate E. coli from known source fecal samples. Known source fecal samples should primarily fill gaps identified in other TSSWCB-funded BST projects, as well as, archived known sources identified through subtask 5.5, and gaps identified through subtask 6.3. Approximately three isolates from each fecal sample 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 will be further analyzed using RP for inclusion in the Texas E. coli BST Library. UTSPH-EPRC and AgriLife SCSC will equitably split workload.*

The following actions have been completed during this reporting period:

a. Known source fecal sample collection in the Lampasas and Leon River watersheds has been completed. 155 of the 213 total samples collected were positive for *E. coli* and 701 isolates have been archived, with 430 isolates to be screened by ERIC-PCR. To date, 117 samples (321 isolates) have been screened by ERIC-PCR and 214 isolates have been selected for the local libraries. RiboPrinting has been completed for 144 of these isolates. Completed local libraries will be individually screened for self-validation before isolates are added to the Texas *E. coli* BST Library.

b. Preliminary work has begun to identify cosmopolitan isolates from the Texas E. coli BST Library.

c. Isolates from 110 known fecal samples from the Attoyac Project have been ERIC-RP and are ready for validation and possible inclusion into the Texas *E.coli* BST Library. Additionally, from the Leona Project, 179 known-source fecal samples have been

collected and processed to date, with 135 samples yielding archived *E.coli* that are currently being fingerprinted for library purposes.

d. Version 3-12 of the library now contains 1459 isolates from 1289 source samples.

87% Complete

Subtask 5.2: *UTSPH-EPRC and AgriLife SCSC will quantify species-specific bacteria production (E. coli and Enterococcus) in feces and measure the variability of this production. While bacteria content of feces has been reported in literature for some species and has been summarized in some reports used in Texas bacteria projects, often this information has been limited to fecal coliform. Known source fecal samples from subtask 5.1 should be used.*

The following actions have been completed during this reporting period:

a. UTSPH-EPRC determined *E. coli* densities for five feral hog fecal samples collected from the Leon and Lampasas River watersheds.

b. Kevin Wagner & Terry Gentry discussed the possibility of combining this data and that from related projects (e.g., fate & transport project) for a publication.

c. Agrilife SCSC enumerated *E. coli* from fecal samples processed for downstream library applications, including the Little Brazos River, Attoyac, and Leona projects, given the samples contained sufficient volumes. *Enterococcus* enumerations are to be included on fecal samples processed this quarter.

50% Complete

Subtask 5.3: *Utilizing known source fecal material, AgriLife SCSC and UTSPH-EPRC will utilize the best available bacterial indicators to further develop and refine species-specific bacteria markers for Bacteroidales PCR. Specifically, efforts will be made on markers to 1) differentiate between ruminants (primarily cattle and deer), 2) identify poultry, and 3) differentiate between domestic swine and feral hogs.*

The following actions have been completed during this reporting period:

a. UTSPH-EPRC has been reviewing PCR and high resolution melt analysis data from domestic swine and feral hogs, and from dairy cattle and dairy lagoon samples for further analysis have been identified. DNA sequencing and analysis for selected samples is underway. Further, selected ERIC-PCR amplicons are being evaluated by DNA sequence analysis to evaluate the possibility of identifying host-specific markers.

50% Complete

Subtask 5.4: *AgriLife SCSC and UTSPH-EPRC will coordinate to conduct comparison studies to evaluate differences in E. coli isolate selection using isolation methods EPA 1603, EPA 1604 and the IDEXX methods. Six water samples will be processed using the EPA 1603 and 1604 methods and the IDEXX method. Ten E. coli isolates per sample per enumeration/isolation (total of 180 isolates) will be analyzed using the ERIC-RP. The suitability of utilizing E. coli isolates processed using methods other than EPA 1603 in BST will be determined.*

The following actions have been completed during this reporting period:

a. UTSPH-EPRC used the Texas *E. coli* BST Library to identify the isolates in the method comparison study.

b. Laboratory analysis finished and initial analysis of results completed.

100% Complete

Subtask 5.5: *UTSPH-EPRC and AgriLife SCSC will work to quantify the extent (quantity) and nature (method) of known source library samples that have been collected by out-of-state labs (Harwood @ University of South Florida, Source Molecular Corp., IEH Laboratories & Consulting Group, Ellender @ University of Southern Mississippi) and other in-state labs (Brinkmeyer @ TAMU-Galveston) for Texas BST projects. To the extent practical and appropriate, UTSPH-EPRC and AgriLife SCSC will work to incorporate known source library samples from these 3rd party labs into the Texas E. coli BST Library. UTSPH-EPRC and AgriLife SCSC and will work with AgriLife BAEN to incorporate known source fecal samples collected through TSSWCB project 07-06 into the Texas E. coli BST Library.*

The following actions have been completed during this reporting period:

a. A total of 85 known source sample from Dr. Karthikeyan's lab at TAMU have been incorporated.

50% Complete

Subtask 5.6: *TWRI, UTSPH-EPRC and AgriLife SCSC will collaborate to 1) expand and update on the list of BST-related R&D activities identified by the Task Force, and 2) prioritize the updated list of BST-related R&D activities.*

The following actions have been completed during this reporting period:

a. Preliminary discussions on this task have been held. Attempts are being made to plan a conference call to discuss these issues next quarter.

20% Complete

TASK 6: BST workshop and state of the science conference delivery and development

Subtask 6.1: *AgriLife SCSC and UTSPH-EPRC 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 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. Workshop on Bacterial Detection and Tracking was presented at the National Water Conference in Portland, OR, 22 May 2012. Kevin Wagner & Terry Gentry co-chaired the workshop and also gave BST presentations along with Emily Martin.

b. Emily Martin submitted abstract for presentation on "Bacterial Source Tracking (BST) in Action: Utilization of BST Toolbox Approach in Texas Watersheds" at the 20th Annual Nonpoint Source Monitoring Workshop in Tulsa, OK, 14-17 October 2012.

c. Conference proceedings from the statewide BST workshop are being completed and will be distributed next quarter.

95% Complete

Subtask 6.2: *TWRI, AgriLife SCSC and UTSPH-EPRC will cooperate to establish a Conference Planning Team that serves as the advisory committee for planning a statewide BST workshop (subtask 6.4) and agency specific meetings. Planning activities will include setting meeting agendas, providing*

information for the development of meeting materials and identifying invited speakers for the statewide BST workshop.

The following actions have been completed during this reporting period:

a. Task complete.

100% Complete

Subtask 6.3: *TWRI will work to coordinate BST meetings with targeted agencies (TCEQ, TDA, TPWD, GLO, DSHS, USEPA, and selected river authorities). The intent of these meetings is further the understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the validity of BST in general, and methods and the Texas E. coli BST Library particularly.*

The following actions have been completed during this reporting period:

a. No new activity to report this quarter.

90% Complete

Subtask 6.4: *TWRI will coordinate the planning of a statewide BST workshop to be held in Austin in summer/winter 2011/2012. The purpose of this workshop will be to 1) highlight the extent of BST work that has been and is being conducted in the state, 2) discuss the scientific advances and improvements in the application of BST, and 3) identify research needs to further the science of BST. TWRI will handle all meeting logistics, speaker invitations, meeting materials preparation and advertising/promotion of the meeting.*

The following actions have been completed during this reporting period:

a. Task complete.

100% Complete

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

IV. Projected Work for Next Quarter

- **i.** meeting on reconciliation of BST and modeling
- **ii.** Completion of known-source isolate analysis and addition to library
- **iii.** Completion of report on BST work conducted in Texas to date
- **iv.** Print and distribute BST conference proceedings
- **v.** Distribute informational flyers to selected parties on the merits of BST