Moving Forward

For future WPP and TMDL development projects, an assessment phase using a "toolbox" approach is recommended. The assessment phase should include targeted monitoring of suspected pollution sources, use of library-independent and dependent methods to identify the presence of domestic sewage pollution and screening of water isolates from the new watershed against the existing library to determine the need for collection of local source samples and expansion of the library.

Decision on which method to utilize can be assisted with the use of the matrix provide in Chapter 2 of the EPA Microbial Source Tracking Guide. It is critical to follow the same analytical protocols for comparability of BST data sets.

The state BST laboratories (UTSPH – El Paso Regional Campus; Texas A&M Soil and Aquatic Microbiology Laboratory) can provide detailed BST protocols. In addition, the sharing of bacterial isolates and BST data between the state laboratories and others is welcomed.

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Texas Water Resources Institute *make every drop count*

BST

Bacterial Source Tracking

UTHealth The University of Texas Health Science Center at Houston | School of Public Health

Soil Water TSSWCB

Texas Water Resources Institute *make every drop count*

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Developed by the Texas Water Resources Institute and funded through a State General Revenue nonpoint source grant from the Texas State Soil and Water Conservation Board

Cover photo: © Lynn McBride EM-110

An introduction for laboratories and public agencies to the foremost tool for identifying sources of fecal pollution

The Need

According to the 2010 Texas Integrated Report, there are 303 bacterially impaired waterbodies in Texas. Nonpoint sources (NPS) of pollution greatly affect water quality. Identifying and assessing sources of fecal pollution is a key component in effectively implementing a NPS pollution management program. Proper evaluation of these sources is needed to properly assess risk in contact recreation, target best management practices, and develop effective watershed protection plans (WPPs) and bacterial total maximum daily loads (TMDLs).

Genotypic (molecular) tools appear to hold promise for BST, providing the most conclusive characterization and level of discrimination for isolates. Of the molecular tools available, automated ribosomal ribonucleic acid (RNA), gene fingerprinting (RiboPrinting), repetitive element polymerase chain reaction (rep-PCR), and pulsed-field gel electrophoresis (PFGE) are emerging as a few of the versatile and feasible BST techniques.

BST Technologies

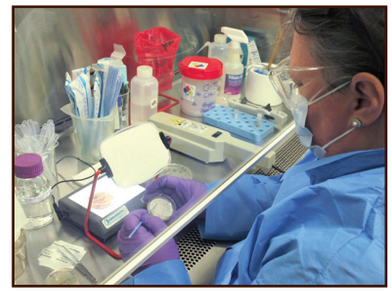
The premise behind BST is that genetic and phenotypic tests can identify bacterial strains that are host-specific so that the original host animal and source of the fecal contamination can be identified. Often *E. coli* or *Enterococcus* spp. are used as the bacteria targets in BST, as this provides a direct link with water quality standards, which are usually based on one of these two indicators. The technologies used for BST have evolved greatly in the past few years.

Identification libraries consisting of thousands of isolates obtained from thousands of animal and human fecal samples collected in different geographical regions of Texas have already been established for ERIC-PCR, PFGE, RiboPrinting, CSU and KB-ARA patterns. In addition, several thousand more *E. coli* isolates from source samples have been archived and are available to researchers.

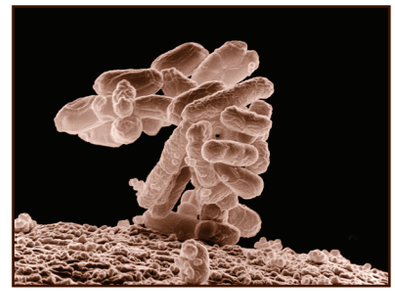
About the Texas *E. coli* BST Library

The Texas *E. coli* BST library currently contains 1,393 *E. coli* isolates obtained from 1,201 different domestic sewage, wildlife, livestock and pet fecal samples. Isolates were selected after screening several thousand isolates from nine different studies throughout Texas.

Library development is one of the most costly components of BST studies. Currently, Dr. George Di Giovanni, at the University of Texas School of Public Health – El Paso Regional Campus, and Dr. Terry Gentry, at the Texas A&M University Soil and Aquatic Microbiology Laboratory, are cross-validating the libraries generated in Texas BST studies in an attempt to explore issues of geographical and temporal stability of BST libraries, refine library isolate selection and determine accuracy of water isolate identification.



By selecting *E. coli* source isolates that are correctly identified from multiple watersheds, the BST library hopes to find more geographically stable and host-specific isolates, resulting in more accurate source tracking. Library-independent methods are also currently being explored, based on Texas Commission on Environmental Quality and Texas State Soil and Water Conservation Board Bacterial Total Maximum Daily Load Task Force recommendations.



Relative comparison of several bacterial source tracking techniques

Technique	Target organism(s)	Basis of characterization	Accuracy of source identification	Size of library needed for water isolate IDs	Capital cost	Cost per sample (reagents and consumables only)	Ease of use	Hands on processing time for 32*** isolates	Time required to complete processing 32 isolates
ERIC-PCR	*E. coli* and *Enterococcus* spp.	DNA fingerprint	Moderate	Moderate	$20,000 ($15,000 BioNumerics software, $5,000 equipment)	$8	Moderate	3 h	24 h**
RP	*E. coli* and *Enterococcus* spp.	DNA fingerprint	Moderate	Moderate	$115,000 ($100K RiboPrinter, $15K BioNumerics software)	$40	Easy	1 h	24 h
PFGE	*E. coli* and *Enterococcus* spp.	DNA fingerprint	High	Large	$30,000	$40	Difficult	10 h	5 days
KB-ARA	*E. coli* and *Enterococcus* spp.	Phenotypic fingerprint	Moderate*	Moderate	$35,000	$15	Easy	3 h	24 h**
CSU	*E. coli* and *Enterococcus* spp.	Phenotypic fingerprint	Moderate	Moderate	$15,000	$10	Easy	4 h	24 h**
Bacteriodales PCR	Bacteriodales species	Genetic marker presence or absence (not quantitative)	Moderate to high for only human, ruminant, horse, and pig sources	N/A	$5,000	$8	Easy to moderate	3 h	8 h**
***E. faecium* esp marker**	*E. faecium*	Genetic marker presence or absence (not quantitative)	High for only human	N/A	$8,000	$8 to $12	Easy to moderate	3 to 6 h	8 to 24 h**
ERIC-RP	*E. coli*	DNA fingerprints	Moderate to high	Moderate	$120,000	$48	Moderate	4 h	24 h
ERIC-ARA	*E. coli*	DNA and phenotypic fingerprints	Moderate to high	Moderate	$55,000	$23	Moderate	6 h	24 h
ARA-CSU	*E. coli* and *Enterococcus* spp.	Phenotypic fingerprints	Moderate to high	Moderate	$50,000	$23	Easy to moderate	7 h	24 h

†A manual ribotyping version is also used by some investigators (i.e. Dr. M. Samadpour with IEH Laboratories and Consulting Group in Seattle), but no detailed information is available for comparison. ‡A variation of this technique using replica plating and +/- scoring of growth on media with different concentrations of antibiotics, called ARA, has been used extensively in Virginia for TMDLs. *This technique is better for distinguishing broader groups of pollution sources. For example, "wildlife" and "livestock" as opposed to "avian wildlife", "non-avian wildlife," "cattle," etc. **With sufficient personnel, up to approximately 150 isolates can be analyzed in 24 h. ***Thirty two isolates selected for comparison because it is the maximum throughput per day of the RiboPrinter, which is the only automated system described.