Notes on Microbial Source Tracking

Prepared for the Nolan Creek/South Nolan Creek Watershed Protection Plan Advisory Committee by the Texas Institute for Applied Environmental Research (June 2016)

Microbial source tracking (MST), which is also often referred to as bacteria source tracking (BST), is a set of methods used to aid in determining the host or source of bacteria (or a broader group of microorganisms) based on the presence of genetic material or "markers." These tests typically aim to separate human from non-human sources and some markers can differentiate among certain animal types. More advanced tests are being developed that evaluate the fuller microbial community, but these tests are still largely experimental.

Deciding Whether to Use MST

A Tiered Approach

Because MST can be quite costly, EPA recommends a tiered approach. Depending on the type of MST test implemented, costs can range from about $400 per sample evaluating a single marker to several thousand dollars per sample if trying to evaluate the full microbial community. Studies, thus, can range from $50,000 to $500,000 or more depending on the scale, type of test, and desired certainty, which is related to the number of samples. Targeted monitoring and watershed surveys are recommended first to identify and track bacteria sources. If there is sufficient uncertainty still regarding sources, then MST may supplement these other approaches.

Types of MST

Single-Marker Methods – Generally used to identify human versus non-human sources using qPCR to target *Bacteroidales* bacteria. *Bacteroidales* is an order of bacteria commonly found in feces with subgroups that appear to be host specific allowing the development of "markers" for specific species.

- Human Markers: HF183 Taquman & HumM2
- Non-Human Markers: Dog, Cow/Ruminant, Pig, Horse, Gull, Birds, Geese, Deer & Beaver

Microbial Community Analysis (MCA) – considered experimental, may require development of a library of sources if single-source markers have not yet been developed for suspected fecal sources. The use of MCA has the potential of providing a measurement of the entire microbial community, but currently is very expensive and must be done in cooperation with a research facility. A type of MCA was done for the Lampasas BST study and involved a collection of fecal samples from known sources for comparison (http://leon-lampasasbst.tamu.edu/).

Study Design Considerations for MST

What question is to be answered by MST? This must be carefully considered as it impacts the scope of the study as well as its cost and the likelihood of obtaining meaningful results.

Human bacteria sources carry a higher risk to public health than non-human fecal sources, so if the presence of human-associated bacterial sources is in question, then maybe only a single-marker method confirming human sources is needed.

If human bacteria sources are not considered dominant, then the next step suggested is to assess if available non-human markers can address the presence/absence of dominant sources. Choosing the right non-human bacteria markers to evaluate for is important as the more markers assessed, the more expensive the analysis.

Other questions:

- How many samples should be collected?
- Which locations should be sampled?
- When should sampling take place?
- Which analytical methods should be used?
- What level of redundancy of markers is required?

Limitations of MST

False Negatives – failure to detect a target that is actually present in a sample ("absence of detection" does not equal "detection of absence")

- Inadequate number of samples (It takes only a few samples to prove presence of a source, but many more to prove absence.)
- Dilution of source water – detection limits very low
- Inhibition of qPCR or interference by other substances in the sample resulting in underestimation of target DNA
- Degradation of target DNA via decay and aging may lead to negative results (sunlight, predation, adsorption to particles and subsequent settling out of the water column)

False Positives – detecting a target when it is actually not there. Occurs much more rarely than false negatives but is a concern due to the economic consequences of potentially remediating a falsely identified source.

- Generally occurs when a host-associated microbe comes from a non-target host (e.g., gulls feeding on biosolids from wastewater treatment plant have tested positive for human markers).

Source Allotment - Markers provide presence/absence but not absolute percent contributions of bacteria from identified sources. Results can indicate relative dominance or rank order, which is often inferred by the frequency with which certain matches occur.

Source Resolution – Only a limited number of sources can currently be identified using existing markers.

References:

Gregory, L., E. Casarez, J. Truesdale, G. Di Giovanni, R. Owen, J. Wolfe. 2013. Bacterial Source Tracking to Support the Development and Implementation of Watershed Protection Plans for the Lampasas and Leon Rivers. Texas Water Resources Institute Technical Report 442. Available online at: http://leon-lampasasbst.tamu.edu/reports/2013/tr442.pdf (link verified 08 June 2016).

Griffith, J.F., B.A. Layton, A.B. Boehm, P.A. Holden, J.A. Jay, C. Hagedom, C.D. McGee, and S.B. Weisberg. 2013. The California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches. Southern California Coastal Water Research Project, Technical Report 804. Available online at: http://www.waterboards.ca.gov/water_issues/programs/beaches/cbi_projects/docs/sipp_revised.pdf (link verified 08 June 2016).

Mauricio, L. 2014. Finding your bacteria sources: Microbial Source Tracking. Presentation given at the Clean Waters Initiative (CWI) Workshop: Microbial Source Tracking, Houston, Texas July 17, 2014. Available online at: https://www.h-gac.com/community/water/cwi/past-workshops/documents/Source%20Molecular%20July%2017%20CWI%20Workshop%20Larenas.pdf (link verified 08 June 2016).

Southern California Coastal Water Research Project (SCCWRP). 2012. Microbial Source Tracking & Identification Fact Sheet. Available online at: http://ftp.sccwrp.org/pub/download/DOCUMENTS/FactSheets/SourceIDFactSheet_web.pdf (link verified 08 June 2016).

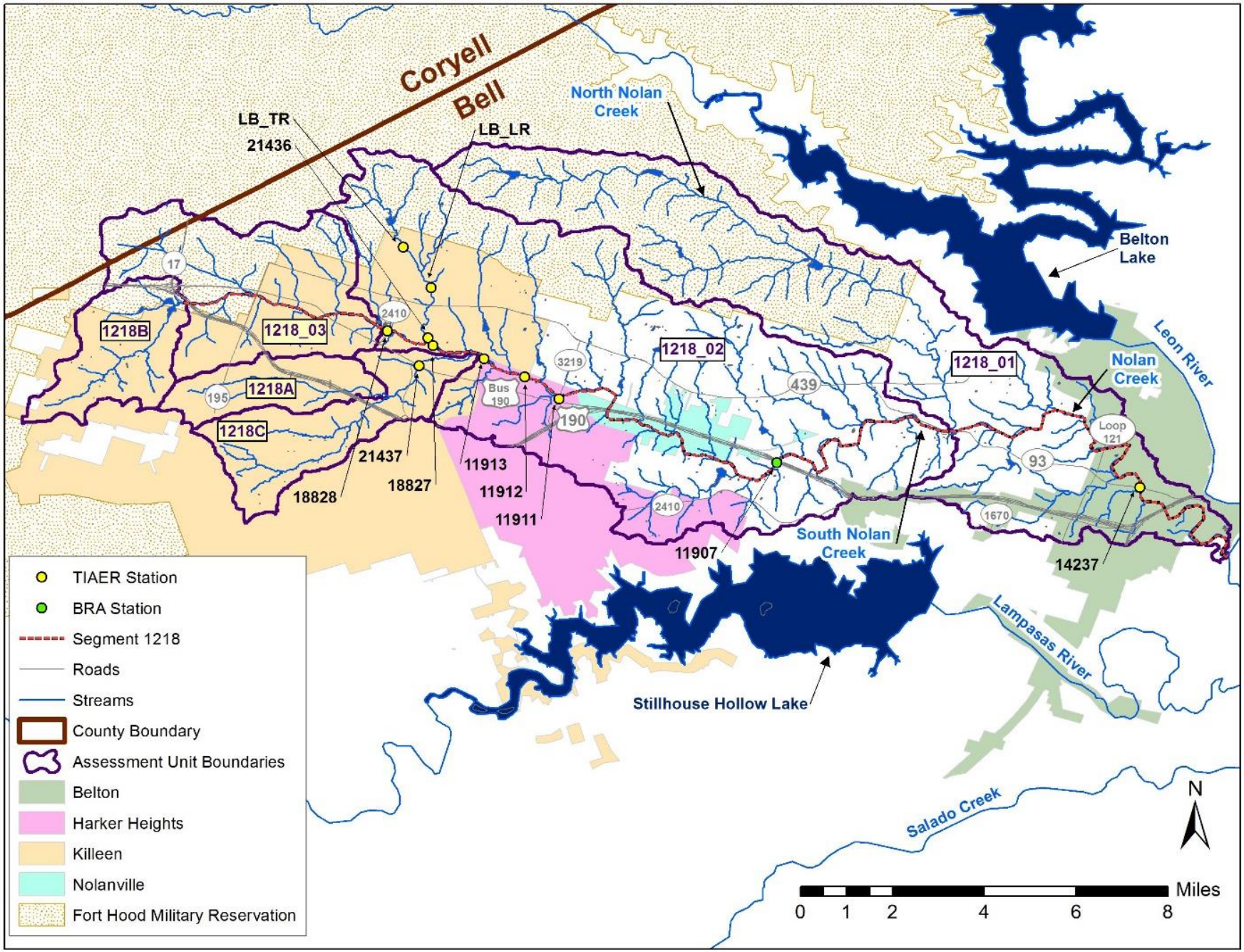
Tetra Tech and Herrera Environmental Consultants. 2011. Using Microbial Source Tracking to Support TMDL Development and Implementation. Prepared for USEPA, Region 10 Watersheds Unit, Seattle, WA. Available online at: https://www3.epa.gov/region10/pdf/tmdl/mst_for_tmdls_guide_04_22_11.pdf (link verified 08 June 2016).

USEPA. 2005. Microbial Source Tracking Guide Document. EPA/600-R-05-064, USEPA, Office of Research and Development, Cincinnati, OH. Available online at: http://twri.tamu.edu/docs/bacteria-tmdl/epa%20mstguide%206-05.pdf (link verified 08 June 2016).

PREPARED IN COOPERATION WITH THE TEXAS COMMISSION OF ENVIRONMENTAL QUALITY AND U.S. ENVIRONMENTAL PROTECTION AGENCY

The preparation of this document was financed through grants from the U.S. Environmental Protection Agency through the Texas Commission on Environmental Quality, Nonpoint Source Grant Program for Project 582-16-60281.

Proposed stations to be monitored by TIAER for the Nolan Creek/South Nolan Creek WPP project. Station 11907 is monitored by the BRA under the Clean Rivers Program.



Proposed stations to be monitored by TIAER for the Nolan Creek/South Nolan Creek WPP project. Station 11907 is monitored by the BRA under the Clean Rivers Program.

TCEQ Station or Temp. ID	Site Description	Rationale for Selection	Latitude	Longitude
18828	South Nolan Creek at 38th St in Killeen	Represents most upstream location of AU 1218_02 with background concentrations generally below PCR criterion for bacteria. Located just below Bell County WCID1 Main Plant WWTF discharge. Of note, the City of Killeen opened Mickey's Dog Park just above this location in June 2015.	31.108091	-97.702156
LB_TR	Long Branch at Tripp Trail in Killeen	Located below reservoir along upper third of Long Branch. Station added to aid in isolating sources on Long Branch.	31.134587	-97.697216
LB_LR	Long Branch at Lake Road in Killeen	Located just above Long Branch Park in Killeen below the confluence with an unnamed tributary on Long Branch. Station added to aid in isolating sources on Long Branch.	31.12176	-97.688445
21436	Long Branch just upstream of crossing of South Nolan Creek at Twin Creek Dr in Killeen	Considered a potential contributing source based on increasing concentrations noted between stations 18828 and 18827.	31.105946	-97.689364
18827	South Nolan Creek at Twin Creek Dr in Killeen	Elevated bacteria concentrations indicated at this location downstream of confluence of Long Branch, a major tributary to South Nolan Creek.	31.103470	-97.687851
21437	Little Nolan Creek off US 190 in Killeen	Considered a potential contributing source based to South Nolan Creek between stations 18827 and 11913. Little Nolan indicated to have elevated bacteria concentrations in the 2014 Texas Integrated Report.	31.097143	-97.692268
11913	South Nolan Creek at Roy Reynolds Road in Killeen	Elevated concentrations indicated at this location on South Nolan just after confluence of Little Nolan Creek.	31.099382	-97.671748
11912	South Nolan Creek at Amy Lane in Harker Heights	Located between station 11913 and 11911 where increases in bacteria are occurring.	31.09361	-97.6589
11911	South Nolan Creek at FM 3219 in Harker Heights	Located below WWTF discharge associated with Harker Heights. Elevated bacteria concentrations noted between stations 11913 and 11911.	31.086666	-97.648056
11907	Nolan Creek at US 190 downstream of Nolanville	Monitoring by Brazos River Authority as part of the Clean Rivers Program	31.06656	-97.5795
14237	Nolan Creek at SH 93 in Belton	Within Yettie Polk Park, a recreational area in Belton. Included to complement quarterly monitoring under the Clean Rivers Program that had been occurring at station 14237 in assessing water quality within AU 1218_01.	31.058743	-97.464989