Recommendations from the Texas Task Force on Bacteria TMDLs

Aaron Wendt
Texas State Soil and Water Conservation Board

Bacterial Source Tracking
State of the Science Conference
February 28-29, 2012
New Braunfels, TX
Water Quality in Texas

- Texas State Soil and Water Conservation Board (TSSWCB)
- Agricultural and Silvicultural Nonpoint Source
- Texas Commission on Environmental Quality (TCEQ)
- Point Source Permitting (WWTF, CAFO, MS4)
- All other forms of Nonpoint Source
Texas Conservation Partnership

Providing Conservation Assistance to Private Landowners for 70+ Years

LOCAL = 216 SWCDs
STATE = TSSWCB
FEDERAL = USDA-NRCS
Why Texas Needed a Task Force

• Texas 303(d) List of Impaired Waters dominated by elevated bacteria related to recreational use and oyster waters use

• Several watershed planning processes (TMDLs or WPPs) on-going with discontented stakeholder groups

• Variety of BST methods/approaches by a number of laboratories had been used in different watershed planning processes
TSSWCB and TCEQ Establish Task Force

- September 27, 2006
- examine approaches other states use to develop bacteria TMDLs
- recommend cost-effective and time-efficient methods and approaches for developing TMDLs and Implementation Plans
- evaluate the variety of models and BST methods available for developing TMDLs and I-Plans, and recommending under what conditions certain methods are more appropriate
- develop a roadmap for further scientific research needed to reduce uncertainty about how bacteria behave under different water conditions in Texas
Task Force Members

- Allan Jones (chair) – Texas Water Resources Institute
- George DiGiovanni – Texas Agricultural Experiment Station
- Larry Hauck – Texas Institute for Applied Environmental Research
- Joanna Mott – Texas A&M University–Corpus Christi
- Hanadi Rifai – University of Houston
- Raghavan Srinivasan – Texas A&M University
- George Ward – University of Texas at Austin
Task Force Report

• http://twri.tamu.edu/what-we-do/finished/bacteria-tmdl/
  – Task Force website with all background information, membership lists, meeting summaries, report drafts, comments rec’d

• June 4, 2007
• TR-341 published by Texas Water Resources Institute
• http://twri.tamu.edu/publications/reports/2009/tr-341/
Task Force Report

• recommended the use of a Three-Tier Approach for bacteria TMDL and Implementation Plan development that is designed to be
  – cost-effective
  – time-efficient
  – scientifically credible
  – accountable to watershed stakeholders

• tiers move through increasingly aggressive levels of data collection and analysis (including BST) in order to achieve stakeholder consensus on needed load reductions and strategies to achieve those reductions
TSSWCB and TCEQ

Adopt Recommendations

• June 29, 2007
• adopted the principles and general process recommended by the Task Force
• directed staff to
  – incorporate the principles of the recommendations into an updated joint-agency TMDL guidance document
  – move diligently to expedite the development of bacteria TMDLs that were paused during the work of the Task Force
  – establish a multi-agency bacteria work group to continue examining the scientific research and development needs identified by the Task Force
What did Task Force say about BST?

• examined use of ERIC-PCR, Ribotyping, PFGE, KB-ARA, CSU, Bacteroidales PCR

• recommended using library-independent methods like Bacteroidales PCR for preliminary qualitative analyses (Tier 2)

• recommended using library-dependent methods if more quantitative data are needed (Tier 3)
## Appendix 4. Table 2. Bacteria TMDLs Under Development in Texas.

<table>
<thead>
<tr>
<th>Project</th>
<th>HSPF</th>
<th>Load Duration</th>
<th>Other Models</th>
<th>Bacteria Source Tracking Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper San Antonio River</td>
<td>☑</td>
<td></td>
<td></td>
<td>ERIC-PCR and RiboPrinting</td>
</tr>
<tr>
<td>Leon River</td>
<td>☑</td>
<td></td>
<td></td>
<td>ERIC-PCR and RiboPrinting</td>
</tr>
<tr>
<td>Peach Creek</td>
<td>☑</td>
<td></td>
<td></td>
<td>ERIC-PCR and RiboPrinting</td>
</tr>
<tr>
<td>Adams and Cow Bayous</td>
<td>☑</td>
<td></td>
<td>RMA2/ACE</td>
<td>No BST</td>
</tr>
<tr>
<td>White Oak and Buffalo Bayous</td>
<td>☑</td>
<td></td>
<td></td>
<td>ARA and CSU</td>
</tr>
<tr>
<td>Lower San Antonio River</td>
<td>☑</td>
<td></td>
<td></td>
<td>ERIC-PCR and RiboPrinting</td>
</tr>
<tr>
<td>Atascosa River</td>
<td>☑</td>
<td></td>
<td></td>
<td>No BST</td>
</tr>
<tr>
<td>Elm and Sandies Creeks</td>
<td>☑</td>
<td></td>
<td></td>
<td>No BST</td>
</tr>
<tr>
<td>Upper Trinity River</td>
<td>☑</td>
<td></td>
<td></td>
<td>Ribotyping (Institute for Environmental Health, Inc., Seattle, WA)</td>
</tr>
<tr>
<td>Guadalupe River above Canyon Lake</td>
<td>☑</td>
<td></td>
<td></td>
<td>Ribotyping (Source Molecular Corporation, Inc., Miami, FL)</td>
</tr>
<tr>
<td>Upper Oyster Creek</td>
<td>☑</td>
<td></td>
<td></td>
<td>Ribotyping (Institute for Environmental Health, Inc., Seattle, WA)</td>
</tr>
<tr>
<td>Copano Bay and Mission and Aransas Rivers</td>
<td>☑</td>
<td></td>
<td>ArcHydro\Monte Carlo Simulation</td>
<td>ARP and PFGE</td>
</tr>
<tr>
<td>Oso Bay and Oso Creek</td>
<td>☑</td>
<td></td>
<td>ArcHydro\SWAT</td>
<td>No BST</td>
</tr>
<tr>
<td>Gilleland Creek</td>
<td>☑</td>
<td></td>
<td></td>
<td>No BST</td>
</tr>
<tr>
<td>Clear Creek</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metropolitan Houston (Brays, Greens, Halls and other Bayous)</td>
<td>☑</td>
<td></td>
<td></td>
<td>ARA and CSU</td>
</tr>
<tr>
<td>WPP – Lake Granbury</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WPP – Buck Creek</td>
<td>☑</td>
<td></td>
<td>TBD</td>
<td><em>E. coli</em>, ERIC-PCR, RP</td>
</tr>
<tr>
<td>WPP – Bastrop Bayou</td>
<td>☑</td>
<td></td>
<td>SELECT, SPARROW, SWAT</td>
<td>No BST</td>
</tr>
<tr>
<td>WPP – Plum Creek</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Relative comparison of several bacterial source tracking techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Acronym</th>
<th>Target organism(s)</th>
<th>Basis of characterization</th>
<th>Previously Used or in Progress in Texas</th>
<th>Used in other states</th>
<th>Accuracy of source identification</th>
<th>Size of library needed for water isolates IDs</th>
<th>Capital cost</th>
<th>Cost per sample (reagents and consumables only)</th>
<th>Ease of use</th>
<th>Hands on processing time for 32*** isolates</th>
<th>Time required to complete processing 32 isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacterial repetitive interspecific consensus sequence polymersase chain reaction</td>
<td>ERIC-PCR</td>
<td><em>Escherichia coli</em> (E. coli) and <em>Enterococcus</em> spp.</td>
<td>DNA fingerprint</td>
<td>Yes (Di Giovanni)</td>
<td>Yes</td>
<td>Moderate</td>
<td>Moderate</td>
<td>$20,000 ($15,000 BioNumerics software, $5,000 equipment)</td>
<td>$8</td>
<td>Moderate</td>
<td>3 h</td>
<td>24 h**</td>
</tr>
<tr>
<td>Automated ribotyping (RiboPrinting)†</td>
<td>RP</td>
<td><em>E. coli</em> and <em>Enterococcus</em> spp.</td>
<td>DNA fingerprint</td>
<td>Yes (Di Giovanni)</td>
<td>Yes</td>
<td>Moderate</td>
<td>Moderate</td>
<td>$40</td>
<td>Easy</td>
<td>1 h</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td>Pulsed field gel electrophoresis</td>
<td>PFGE</td>
<td><em>E. coli</em> and <em>Enterococcus</em> spp.</td>
<td>DNA fingerprint</td>
<td>Yes (Pillai and Lehman)</td>
<td>Yes</td>
<td>High</td>
<td>Large</td>
<td>$30,000</td>
<td>$40</td>
<td>Difficult</td>
<td>10 h</td>
<td>3 days</td>
</tr>
<tr>
<td>Kirby-Bauer antibiotic resistance analysis†</td>
<td>KB-ARA</td>
<td><em>E. coli</em> and <em>Enterococcus</em> spp.</td>
<td>Phenotypic fingerprint</td>
<td>Yes (Mott)</td>
<td>Yes</td>
<td>Moderate*</td>
<td>Moderate</td>
<td>$35,000</td>
<td>$15</td>
<td>Easy</td>
<td>3 h</td>
<td>24 h**</td>
</tr>
<tr>
<td>Carbon source utilization</td>
<td>CSU</td>
<td><em>E. coli</em> and <em>Enterococcus</em> spp.</td>
<td>Phenotypic fingerprint</td>
<td>Yes (Mott)</td>
<td>Yes</td>
<td>Moderate</td>
<td>Moderate</td>
<td>$15,000</td>
<td>$10</td>
<td>Easy</td>
<td>4 h</td>
<td>24 h**</td>
</tr>
<tr>
<td>Bacteroidales polymersase chain reaction</td>
<td>Bacteroidales PCR</td>
<td><em>Bacteroidales</em> species</td>
<td>Genotypic marker presence or absence (not quantitative)</td>
<td>Yes (Di Giovanni)</td>
<td>Yes</td>
<td>Moderate to high for <em>coli</em>, humin, ruminant, horses, and pig sources</td>
<td>Not applicable</td>
<td>$5,000</td>
<td>$8</td>
<td>Easy to moderate</td>
<td>3 h</td>
<td>8 h**</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> surface protein polymersase chain reaction or colony hlys</td>
<td>ERIC and RP 2-method composites</td>
<td><em>E. faecium</em> spp marker</td>
<td><em>E. faecium</em></td>
<td>Yes (Di Giovanni)</td>
<td>Yes</td>
<td>High for <em>coli</em> humin</td>
<td>Not applicable</td>
<td>$8,000</td>
<td>$8 to $12</td>
<td>Easy to moderate</td>
<td>3 to 6 h</td>
<td>8 to 24 h**</td>
</tr>
<tr>
<td>ERIC and RP 2-method composites</td>
<td>ERIC-RP</td>
<td><em>E. coli</em></td>
<td>DNA fingerprints</td>
<td>Yes (Di Giovanni)</td>
<td>No</td>
<td>Moderate to high</td>
<td>Moderate</td>
<td>$120,000</td>
<td>$48</td>
<td>Moderate</td>
<td>4 h</td>
<td>24 h</td>
</tr>
<tr>
<td>ERIC and KB-ARA 2-method composites</td>
<td>ERIC-ARA</td>
<td><em>E. coli</em></td>
<td>DNA and phenotypic fingerprints</td>
<td>Yes (Mott)</td>
<td>No</td>
<td>Moderate to high</td>
<td>Moderate</td>
<td>$55,000</td>
<td>$23</td>
<td>Moderate</td>
<td>6 h</td>
<td>24 h</td>
</tr>
<tr>
<td>KB-ARA and CSU 2-method composites</td>
<td>ARA-CSU</td>
<td><em>E. coli</em> and <em>Enterococcus</em> spp.</td>
<td>Phenotypic fingerprint</td>
<td>Yes (Mott)</td>
<td>Yes</td>
<td>Moderate</td>
<td>Moderate</td>
<td>$50,000</td>
<td>$23</td>
<td>Easy to moderate</td>
<td>7 h</td>
<td>24 h</td>
</tr>
</tbody>
</table>

† A manual ribotyping version is also used by some investigators (i.e., Dr. M. Sambandari 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 *rbcL* scoring of growth on media with different concentrations of antibiotics, called ARA, has been used extensively in Virginia for TMDLA.
* 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.
What did Task Force say about BST?

- Need to clearly define agency/stakeholder expectations for BST and capabilities of BST
- Impact of indicator bacteria survival and regrowth in the aquatic environment, sediment, and soils on BST
- Appropriate level of discrimination of BST results – individual species, human or animal, or some level between
- BST typically identifies only source, not entry pathways of fecal pollution – importance of sampling regime
- In nearly all cases, no single BST method should be solely relied upon
- Laboratory infrastructure for BST work in Texas needs to be expanded for both library dependent as well as library independent methods
What did TF say about Library-Dependent BST?

- Recommend composite library-dependent BST using 1 of 3 combination methods:
  - ERIC-PCR and RiboPrinting (ERIC-RP)
  - ERIC-PCR and KB-ARA (ERIC-ARA)
  - CSU and KB-ARA (CSU-ARA)

- Library development is one of the most costly components of BST
  - most economical to build upon the libraries already established in Texas
  - recommended to use BST methods that will strengthen and expand the current Texas library and follow previously approved SOPs
What did TF say about Library-Independent BST?

• Library-independent methods are cost-effective, rapid and potentially more specific and accurate than library dependent methods

• Concerns regarding geographical stability of markers

• Concerns about the difficulty of interpreting results in relation to water quality standards (i.e., Bacteroidales vs. E. coli)

• Recommend library-independent PCR genetic test for Bacteroidales markers
  – human
  – ruminants
  – horse
  – swine
What did Task Force say about Tier 2 BST?

- conducted in conjunction with the targeted monitoring
- determine if livestock, humans and/or non-domestic animals are contributing bacteria

• Library Independent
  - samples analyzed using PCR genetic test for the Bacteroidales markers for human, ruminants, horse and swine

• Library Dependent (limited)
  - E. coli isolates from water samples analyzed using the Tier 3 methods
  - Compared to previously developed Texas Known Source Library
  - determine the need for development of a local source library
  - confirm that the sources of E. coli and Bacteroidales are comparable
What did Task Force say about Tier 3 BST?

- Library Dependent
  - Use 1 of 3 combination methods
  - ERIC-RP, ERIC-ARA or CSU-ARA
- If Tier 2 BST does not provide 80% identification using existing statewide library, then statewide library needs to be augmented with local known sources
  - Add isolates from known fecal samples (~3 isolates/sample)
- Conduct BST on ambient water samples using the selected combination method
  - Identified to cattle, other livestock, avian and non-avian non-domestic animals, domestic sewage, pet sources, unknown
  - Sources should be expressed as percentages of total isolates with appropriate confidence intervals
What did Task Force say about BST R&D?

• Improve linkages of BST and computer modeling. Models can be validated with BST or vice versa.
• Determine reasonable expectation for the level of source identification by BST
• Refinement of library-independent BST methods and species-specific markers
• Investigate geographic and temporal stability of BST known source libraries
• Define appropriate ambient water sampling protocol to provide desired statistical confidence with BST
Aaron Wendt
Statewide Watershed Planning Coordinator

Texas State Soil and Water Conservation Board

PO Box 658
Temple, TX  76503

(254) 773-2250 ext 232 v
(254) 773-3311 f
awendt@tsswcb.state.tx.us

http://www.tsswcb.texas.gov/

Authorization for use or reproduction of any original material contained in this presentation is freely granted. TSSWCB would appreciate acknowledgement.