

Tracking and Investigating Microbial Sources (TIMS)

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Orange Creek Basin TMDL Working Group

Gainesville Creeks

- **3 primary creek systems in Gainesville (Hogtown, Tumblin, Sweetwater Branch) impaired by elevated levels of fecal coliform bacteria**
- **Fecal coliform bacteria is an indicator of harmful pathogenic organisms in water**
- **Bacteria counts often elevated in urban areas**
- **Conventional Testing cannot distinguish between sources**

GRU Study Purpose

- **Put our wastewater collection system management programs to ultimate test**
- **Help community solve creek problems using best available scientific information**

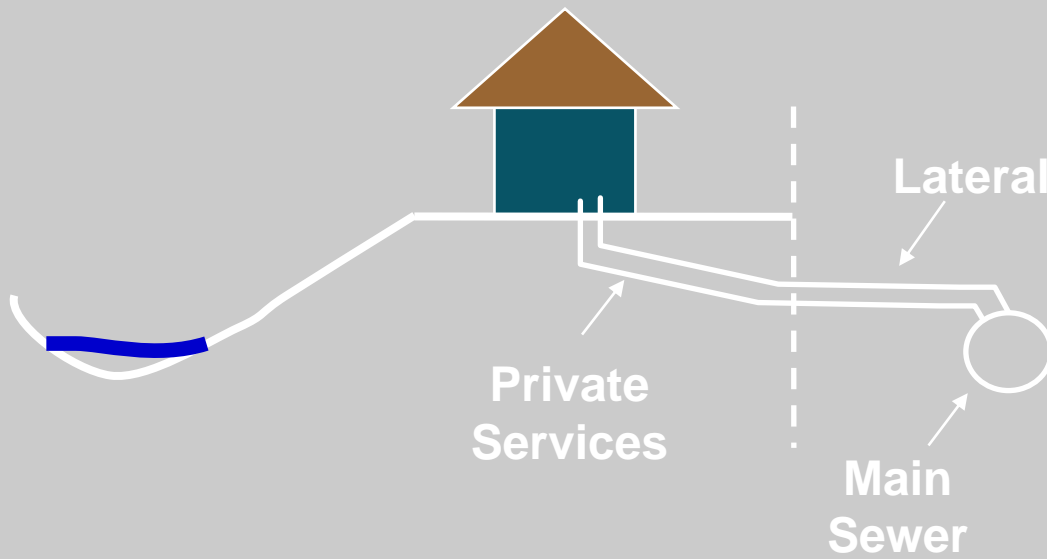
Bacteria Source Tracking

- **Identifying sources of fecal coliform is limited by conventional testing methods that don't reliably distinguish between sources:**
 - **Exfiltration and releases from wastewater collection system**
 - **Malfunctioning septic systems**
 - **Homeless population near creeks**
 - **Domestic animals**
 - **Wildlife**

Wastewater System Sources

- **Wastewater collection system easy target of blame**
 - Easy for public to understand connection
 - Utilities / Agencies are easier focus than scrutiny of private individuals
 - Permit holders - existing regulatory mechanisms to initiate corrective actions
- **But, other potential sources can't be ignored**

Private Systems



Private Collection System

GRU Collection System



Other Creek Side Uses



Animals



Study Scope

- **3 Phase Approach:**
 - **Phase 1: Synoptic Sampling – series of “snapshots” in time of the urban creek system using conventional tests (total coliform, fecal coliform, and enterococcus)**
 - **Phase 2: Detailed Bacterial Source Tracking (BST) Pilot Testing**
 - **Phase 3: Focused BST Sampling Program**
 - **Cost: \$250,000 to \$300,000**

BST Approach from Pilot Testing

- **Test and Confirm with human genetic markers (Enteric Virus, Enterococcus)**
 - Is human marker present?
 - Is human marker persistent?
- **Isolate each bacteria and use genetic Fingerprint (ribotyping)**
 - What is contribution from each source?

Human Markers

- **Human Viruses**
- **Human Enterococcus**
- **Presence / Absence Test**
- **Highly Sensitive – If it is there it will show up.**

Human Markers

- Polymerase Chain Reaction (PCR)
- Detecting presence by multiplying DNA of marker
- If marker present in sample, PCR will multiply it 2^{35} times making detection very sensitive



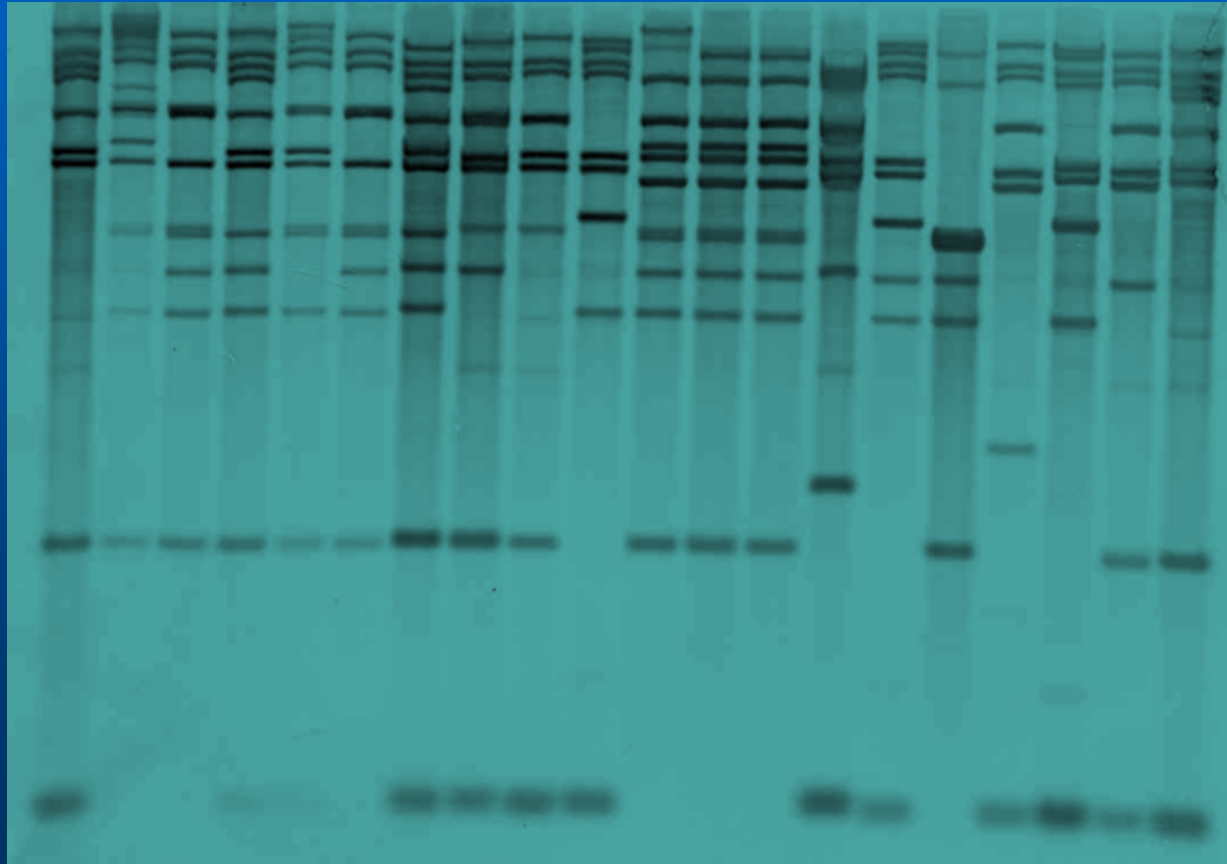
Human Markers

Human Enterococcus	+	+	-	-
Human Virus	+	-	+	-

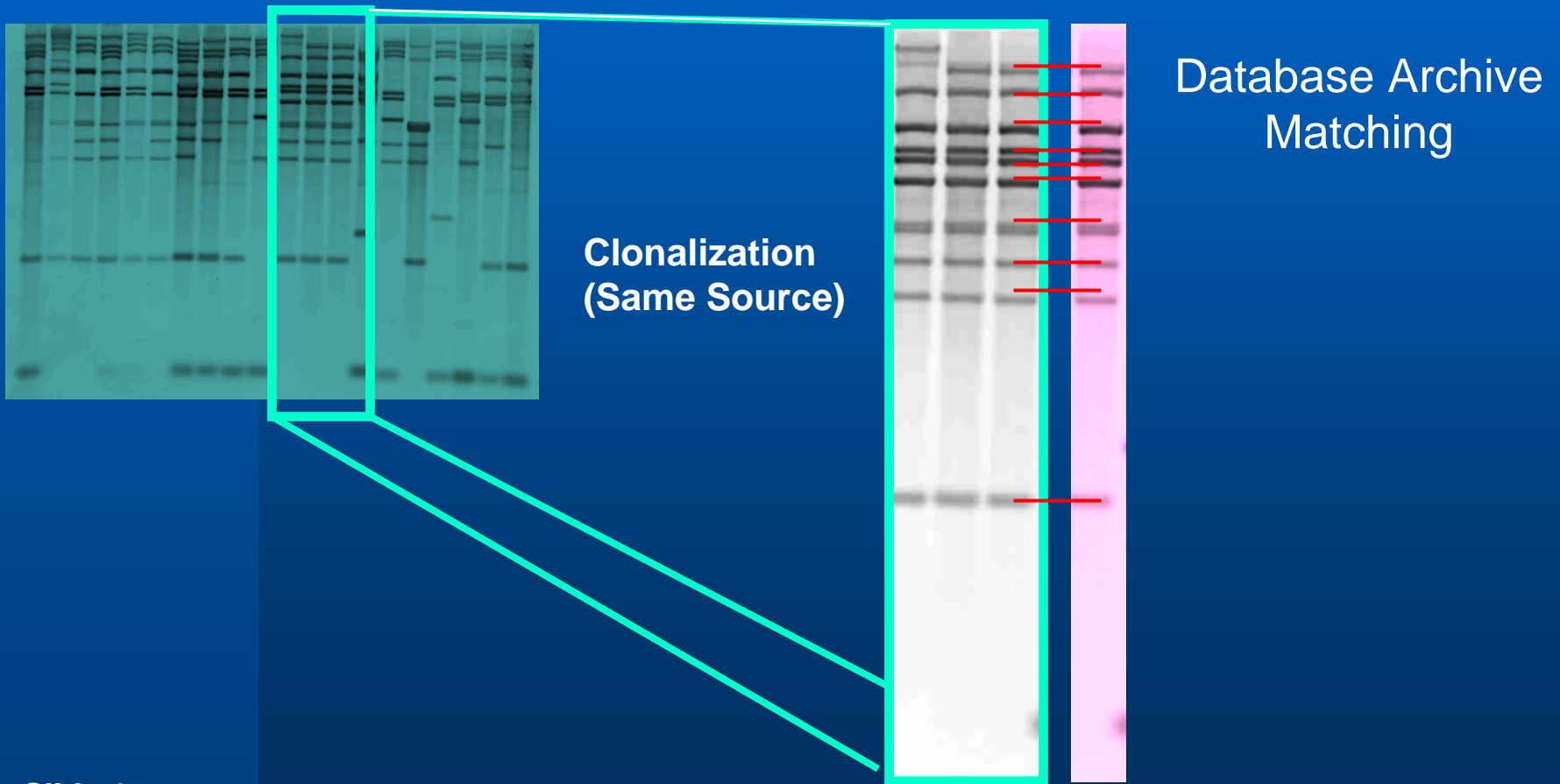
Genetic Fingerprinting (Ribotyping)

- Involves analyzing isolates of e coli
- Provides snapshot of the sample's composition
- Compares isolates to national genetic library database for various species.
- Computer matching is confirmed with human interpretation.
- Analytes are not forced into categories – creates indeterminate category for shared bacteria and isolates not in database.

Genetic Fingerprinting

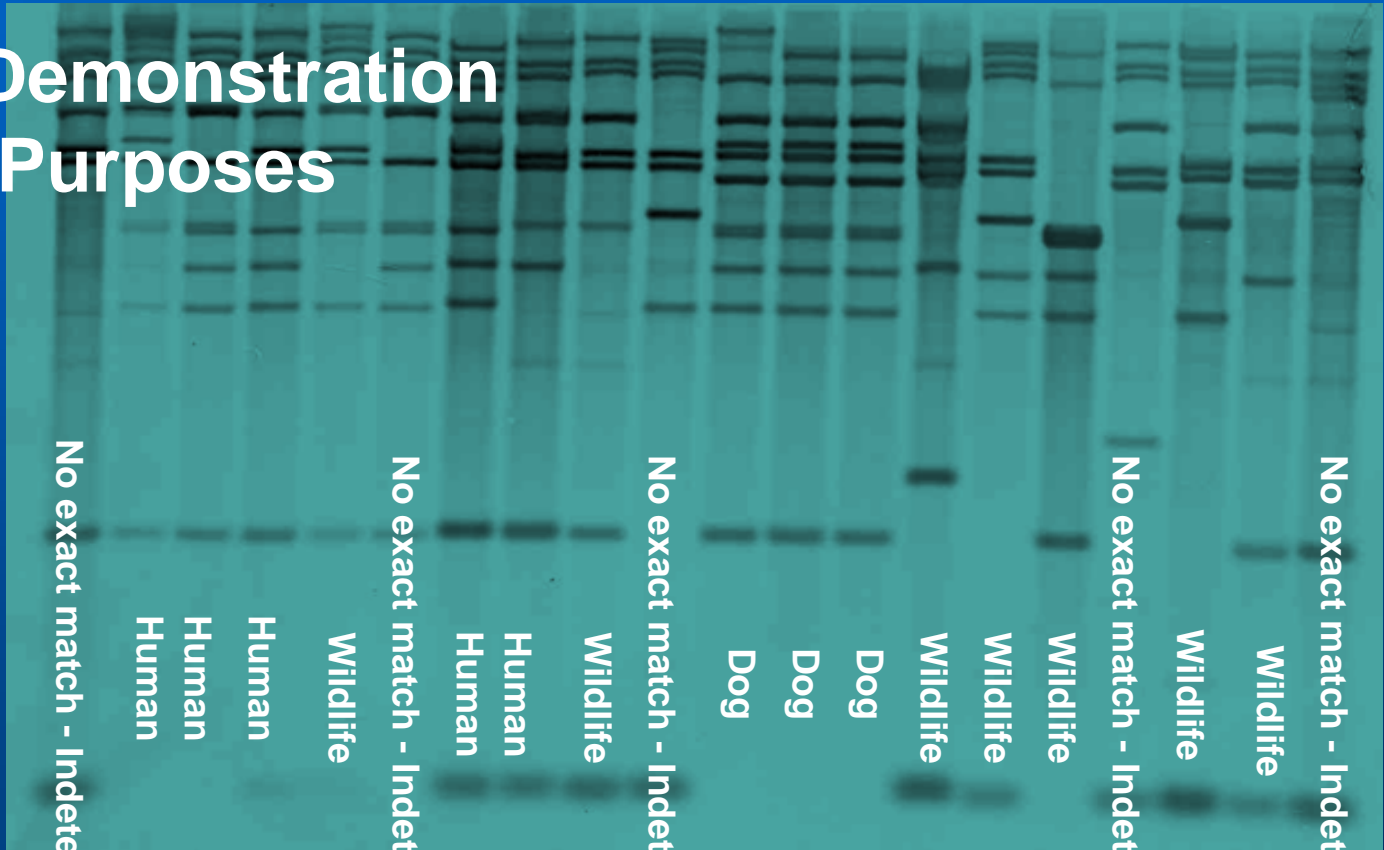


Genetic Fingerprinting



Genetic Fingerprinting

For Demonstration
Purposes



No exact match - Indeterminate

Wildlife

Wildlife

No exact match - Indeterminate

Wildlife

Wildlife

Wildlife

Dog

Dog

Dog

No exact match - Indeterminate

Wildlife

Human

Human

No exact match - Indeterminate

Wildlife

Human

Human

Human

No exact match - Indeterminate

Status

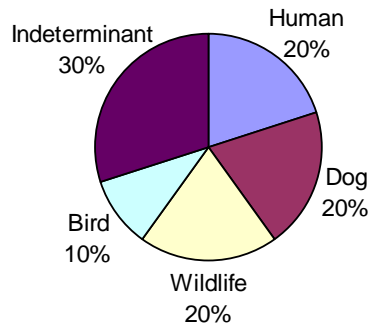
- **Phase 1: 6 of 6 sampling events completed (3 dry weather, 3 wet weather at 43 locations)**
- **Phase 2: Pilot Testing conducted at 3 sampling locations to test best methodologies for achieving study goals - Developed a hybrid approach**
- **Phase 3: 4 events at 10 stations. 1st event complete. Additional stations and events to be coordinated with local DOH septic tank evaluation**

Phase 1 Results

- **Bacteria levels high everywhere most of the time under wet or dry conditions**
- **Fecal coliform to Enterococcus ratios not consistent with wastewater ratios**
- **Creeks are impacted from urban setting**
- **Fecal numbers and past history used to select 3 Phase 2 pilot stations**

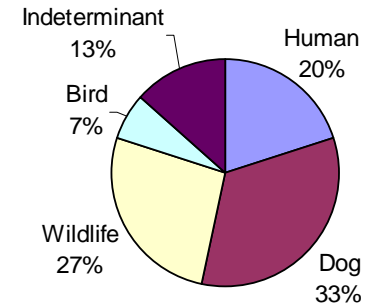
Phase 2 Results

S-7 Sweetwater branch



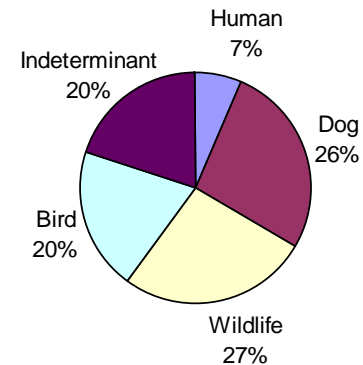
Enteric Virus DNA Marker = Positive
Enterococcus DNA Marker = Positive

S11 Hogtown Creek



Enteric Virus DNA Marker = Negative
Enterococcus DNA Marker = Positive (Faint)

S37 Hogtown Creek



Enteric Virus DNA Marker = Negative
Enterococcus DNA Marker = Negative

Phase 2 Results

- **Developed a multiple method approach.**
- **Human markers are present in results**
- **The preliminary results show that the primary source of fecal coliforms are from non-human sources**

Phase 3 Results

- **Still processing data:**
 - Locations with high fecal counts w/o human marker presence
 - Human markers are more present in the urban areas
 - Added additional human marker (anaerobe)
 - No information on relative contribution of each source yet
- **Coordinating future sampling with DOH septic tank survey**

Conclusion

- **These are the first steps in developing focused solutions. Each stakeholder will have to do their part**
- **Contribution from each human source still not distinguishable, but results can be used to target assessments on based local conditions**
- **Non-human aspects of bacteria will likely need to be addressed to achieve water quality standards**

Credits

- **BST WORK**

- **Bill Dunn and Martha Klein, CH2M Hill (Gainesville)**
- **Troy Scott, Biological Consulting Services of North Florida (Gainesville)**