

Validation and Field Testing of Microbial Source Tracking Methodologies in the Gulf of Mexico

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Premise of the Research

- **MST can be useful for identifying human sources of pollution in Gulf of Mexico waters**
- **MST methods that are validated across the GoM geographic region in several labs using standard operating procedures are desirable for regulatory and public safety applications**
- **Validation must include considerations of sensitivity, specificity, limit of detection, precision, and inter-lab comparability....**

...In Dissimilar Waters Across a Geographic Range



Suwannee River Plume
disc.gsfc.nasa.gov/.../black_water.shtml

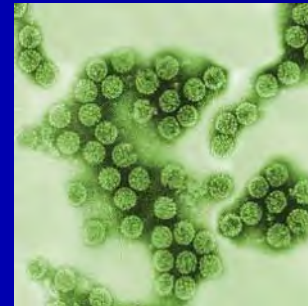
Study Design - Participants

- **3 co-PI labs (USEF, USM, UWF)**
- **Collaborators**
 - **Texas A&M – DigGiovanni**
 - **Texas A&M - Mott**
 - **Nicholls State (LA) – Kilgen & Nathaniel**



Study Design – MST Strategy

- **Library-independent methods**
- **End point (conventional) PCR**
- **Human-associated targets**
 - *Bacteroidetes* w/HF183 primer (Bernhard & Field) 16S rRNA gene
 - *Methanobrevibacter smithii* *nifH* gene
 - Human polyomaviruses BKV and JCV conserved T antigen



Metrics

- **Sensitivity** – ability to detect target when present
- **Limit of detection** – quantitative assessment of sensitivity, i.e. how little can we reliably detect?
- **Specificity** – ability to rule out target when absent



Issues

- **Standardize protocols!**
- **Optimal Concentration/Extraction Procedures**
- **Controls! e.g. extraction blanks**
- **Inhibition of PCR – matrix spikes and test for 16S rRNA or total *Bacteroidetes***



Year 1 Tasks for co-PIs

HF183, *M. smithii*, HPyVs

- **Sensitivity (100%) and limit of detection for sewage spiked in:**
 - **Buffer**
 - **Fresh water (regional)**
 - **Salt water (regional)**
- **Specificity tested against:**
 - **Cattle feces**
 - **Dog feces**
 - **Cat feces**
 - **Bird feces**



Limit of Detection (Sewage)

- **Human-associated *Bacteroidetes* (HF183) could be reliably detected at 10^{-6} dilution (1 million fold)**
- ***M. smithii* could be reliably detected at 10^{-4} to 10^{-3} dilution**
- **HPyVs could be reliably detected at 10^{-4} to 10^{-3} dilution**
- **Water type does not affect LoD**

Specificity

n=>316

- **The human *Bacteroidetes* assay cross-reacted with dog feces (11%) and with chicken feces**
 - Overall specificity = 96%
- **The *M. smithii* assay cross-reacted with 1 cow, 2 dog and 2 seagull samples**
 - Overall specificity = 98%
- **The HPyVs assay showed 100% specificity**

Blind Sample Trials

Co-PIs (UWF, USM) have blind-tested the following samples prepped by concentration onto membrane filter

- **Clones spiked into buffer**
- **Sewage spiked into buffer (500 μl in 500 ml)**
- **Cow feces spiked into buffer**
- **Buffer control**

Just sent: blinds in marine water



Collaborators (Year 2)

- **Texas A&M El Paso and**
- **Texas A&M Corpus Christi have carried out the standard operating procedures (SOP) with clones and sewage.**
- **Next step: limit of detection with sewage and with cloned DNA in plasmids**

Year 2 for Co-PIs Field Validation

- **Workshop Friday determines which method(s) advance to field validation**
- **Sample anthropogenically impacted site vs. relatively unimpacted site(s) over the next year**
- **Test for MST markers and for enterococci**
- **Continue blind samples, including collaborators**

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