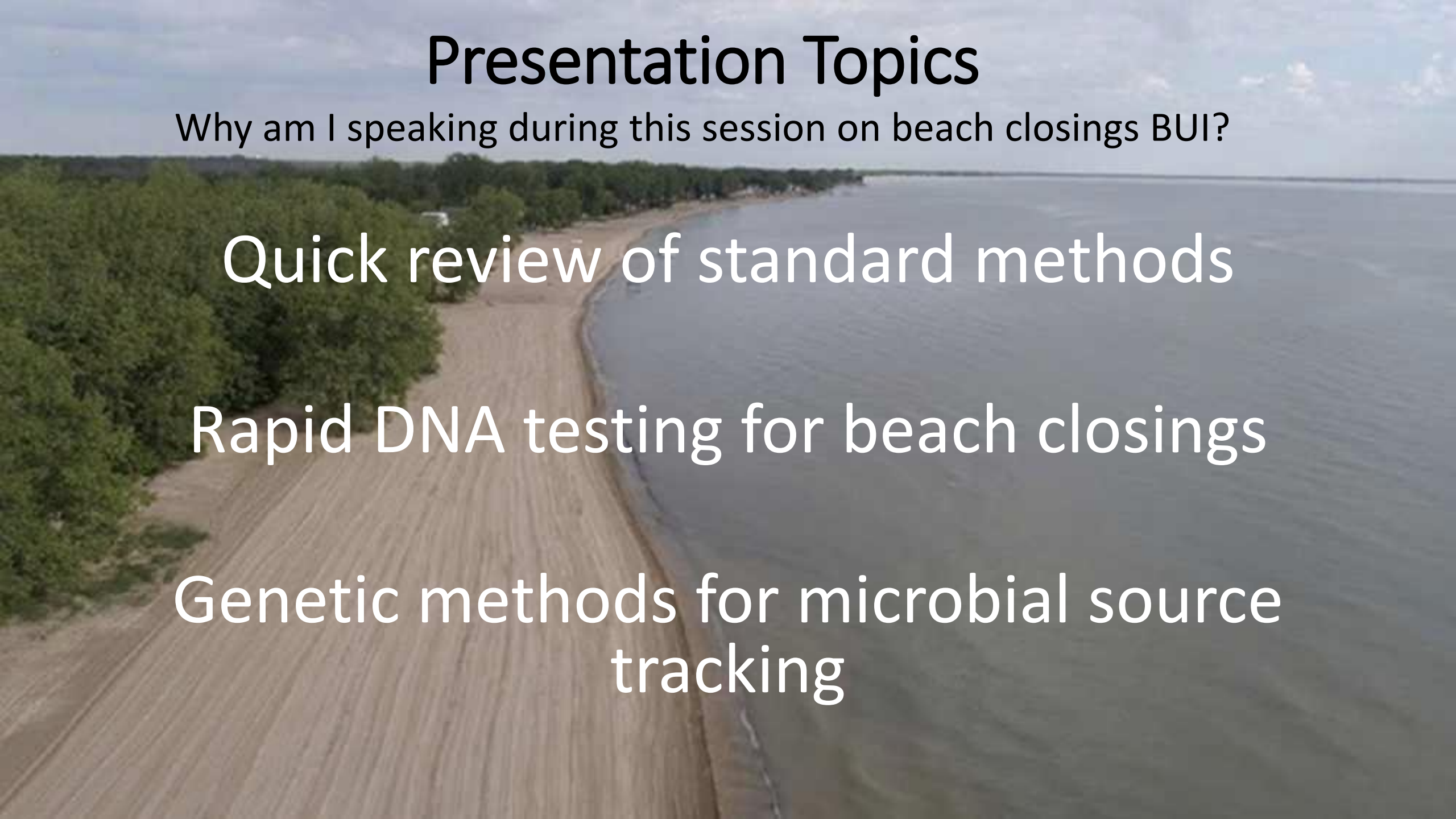
# Using molecular biology tools to investigate fecal contamination

Tami L. Sivy, Ph.D.

Professor of Chemistry

# Presentation Topics

Why am I speaking during this session on beach closings BUI?



Quick review of standard methods

Rapid DNA testing for beach closings

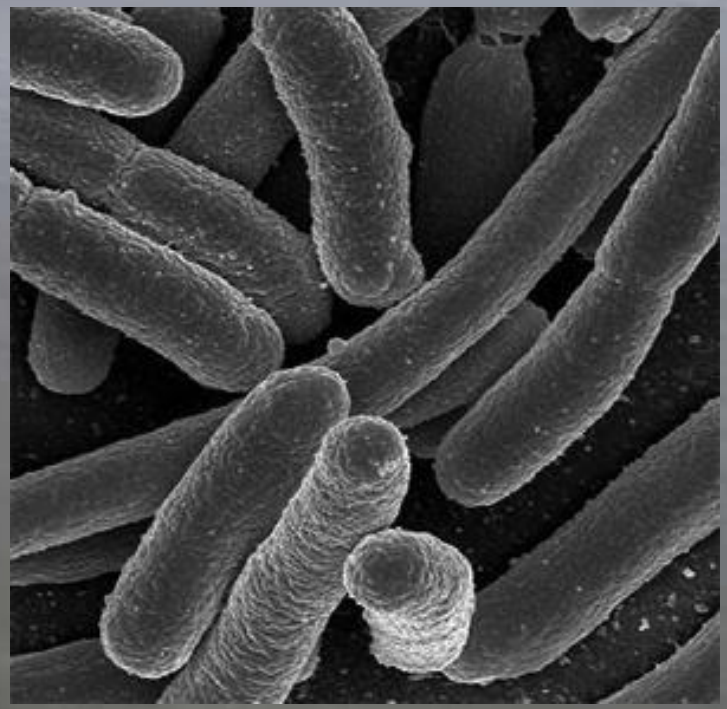
Genetic methods for microbial source tracking

# Fecal indicator bacteria

## Fecal coliforms

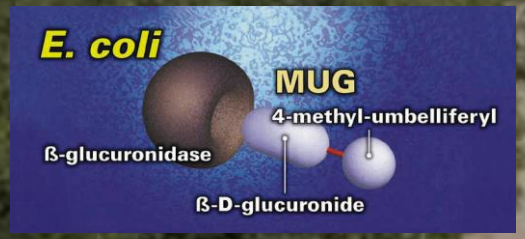
--bacterial indicator of feces contamination

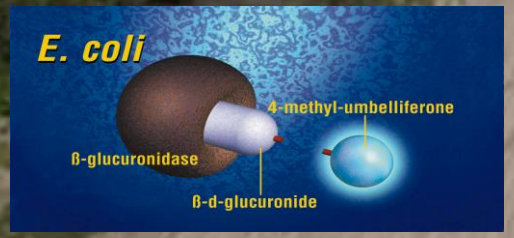
--typically *Escherichia coli* are measured

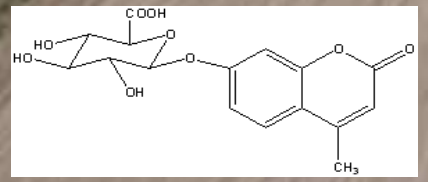


# Standard EPA culture method

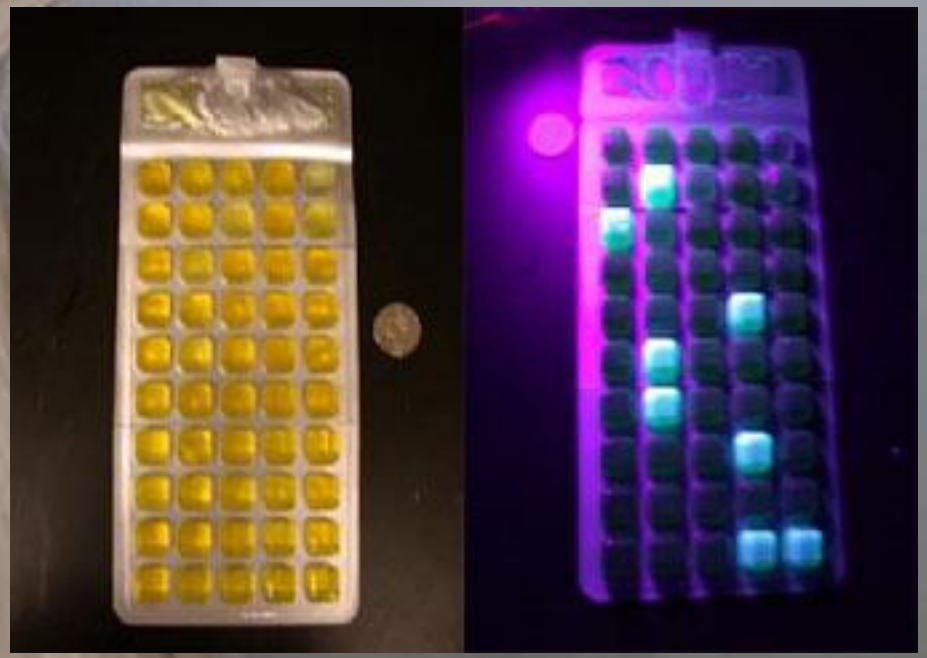
Colilert culture method measures *E. coli* levels after overnight growth



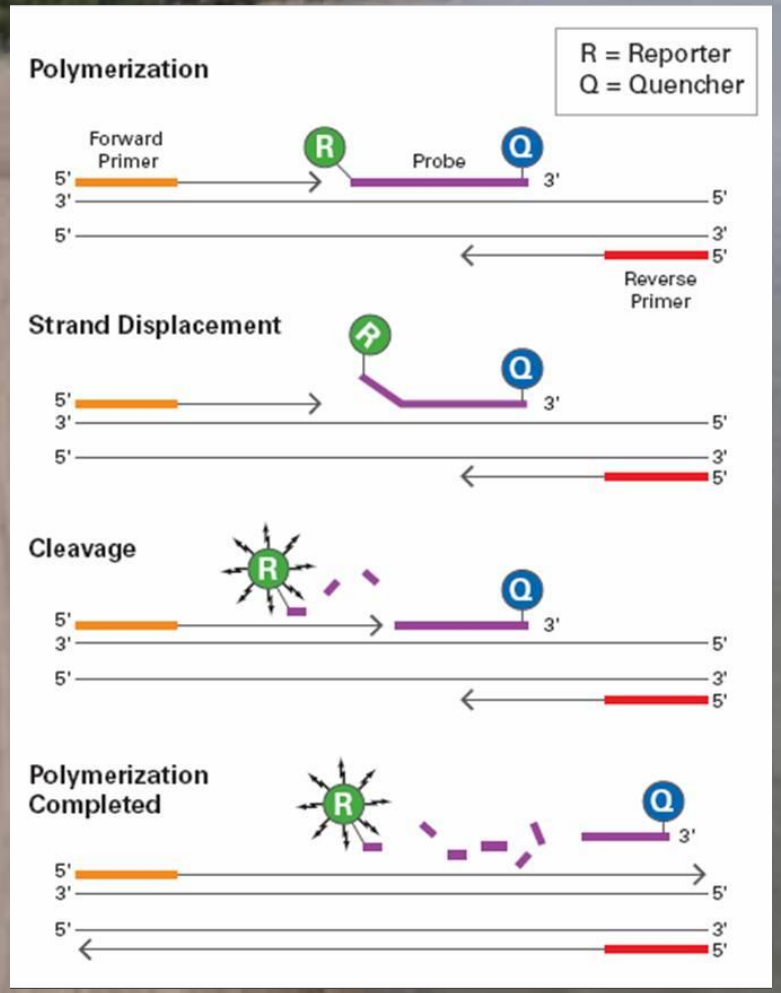

4-Methylumbelliferyl β-D-glucuronide (MUGlcU)



*Allowable level for full body human contact is < 300 CFU/100 mL

# Rapid DNA testing:
# Taqman qPCR relies on a fluorescent reporter

(qPCR: quantitative Polymerase Chain Reaction)




http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/real-time-pcr/taqman-probe-based-gene-expression-analysis.html

# Rapid DNA testing

Collect water sample

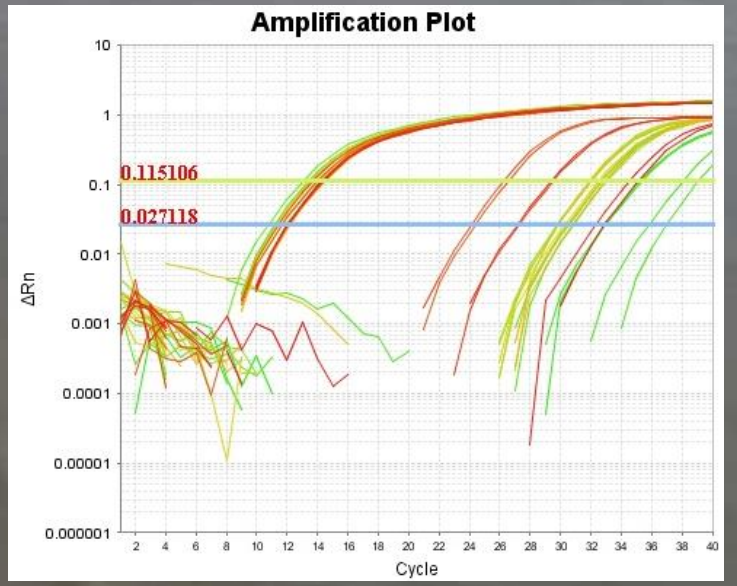
Transport to lab

Filter water sample

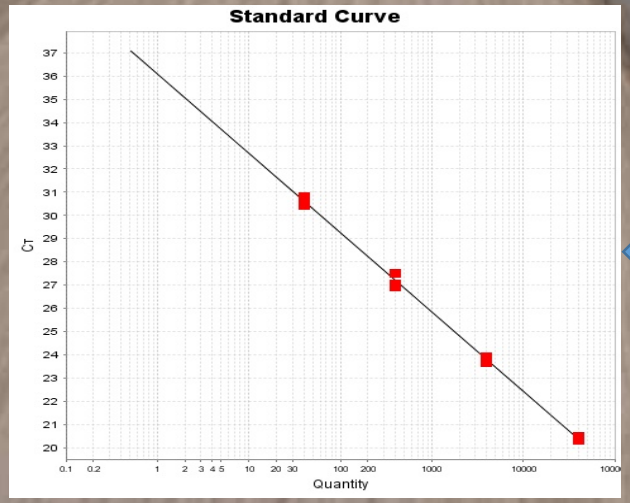
Extract DNA

Run qPCR

Obtain results




Analyze data




Fluorescence $\alpha$ $1/C_T$

$C_T \alpha$ 1/[DNA]

Adapted from MSU figure

# Validation study

21 laboratories –
*E. coli* qPCR method

Standardized protocol, same prescribed reagents, reference and control materials

Master standard curves, positive & negative control data

Standardized data quality acceptance criteria

Water Research
Volume 156, 1 June 2019, Pages 456-464

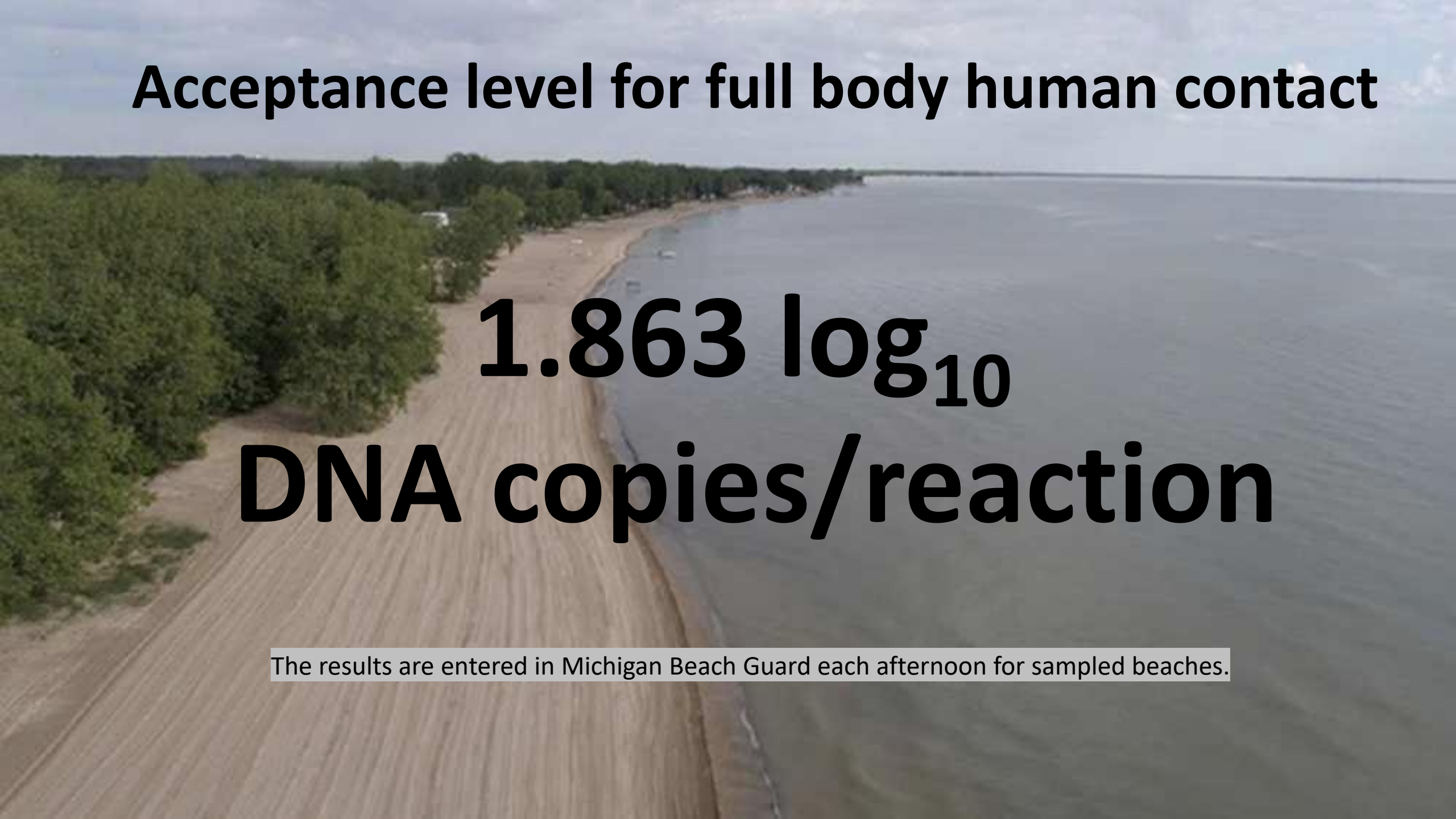
# Standardized data quality acceptance criteria for a rapid *Escherichia coli* qPCR method (Draft Method C) for water quality monitoring at recreational beaches

Mano Sivaganesan [a], Tiong Gim Aw [b], Shannon Briggs [c], Erin Dreelin [d], Asli Aslan [e], Samuel Dorevitch [f], Abhilasha Shrestha [f], Natasha Isaacs [g], Julie Kinzelman [h], Greg Kleinheinz [i], Rachel Noble [j], Rick Rediske [k], Brian Scull [k], Susan Rosenberg [l], Barbara Weberman [l], Tami Sivy [m], Ben Southwell [n], Shawn Siefring [o] ... Richard Haugland [o]

Show more

https://doi.org/10.1016/j.watres.2019.03.011

# Acceptance level for full body human contact



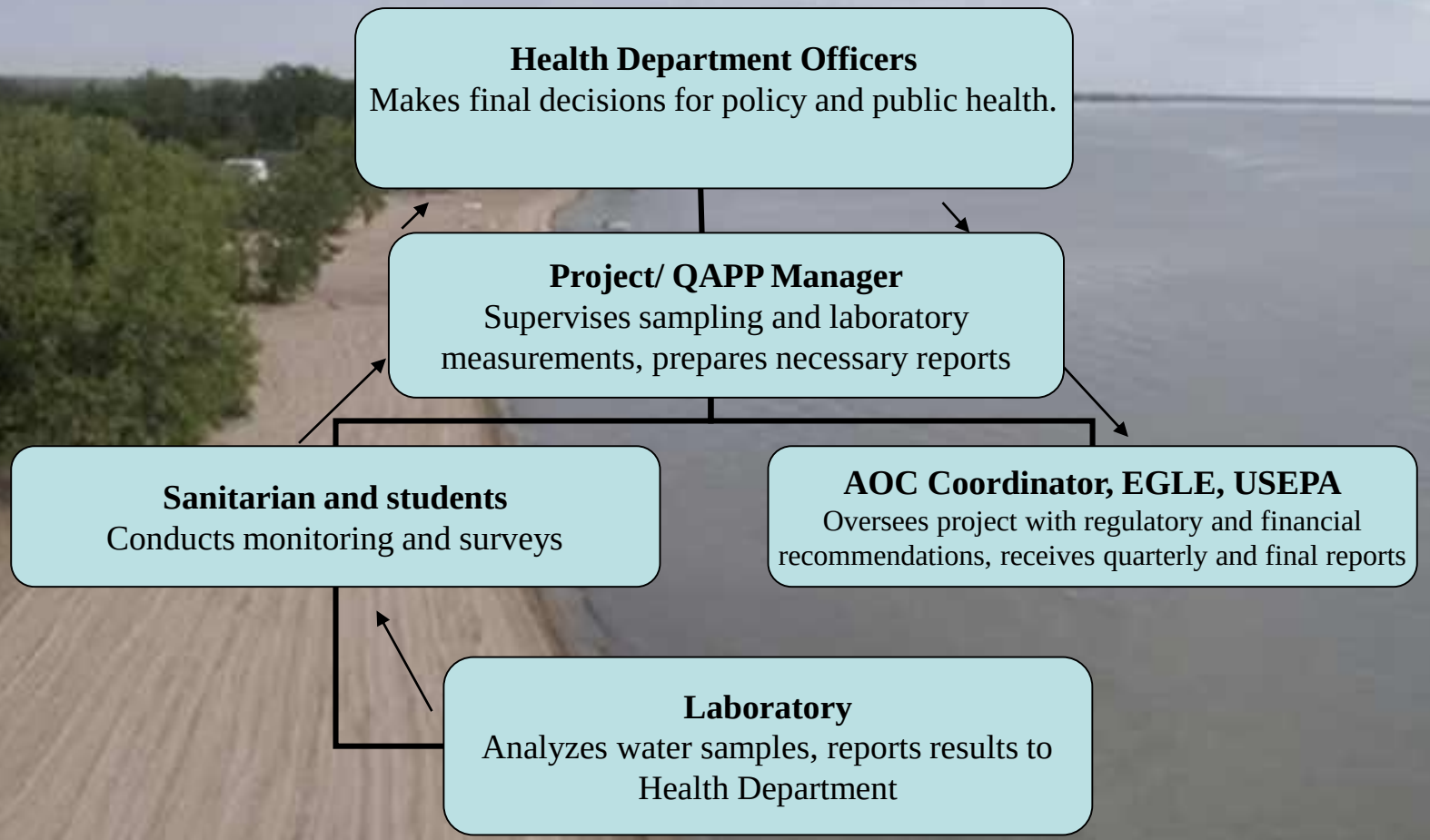
## $1.863\ \log_{10}$ DNA copies/reaction

The results are entered in Michigan Beach Guard each afternoon for sampled beaches.

# qPCR sampling sites now


Map produced by Chris Vandenberg, MDEQ

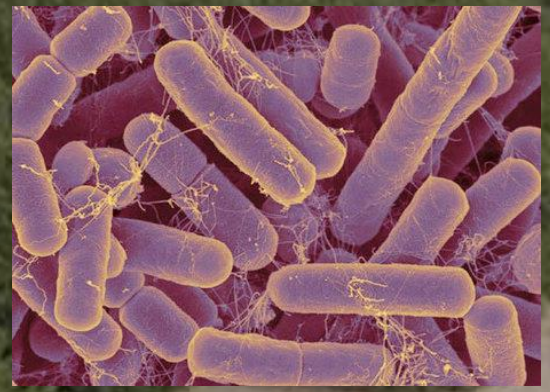
# Microbial Source Tracking project




**The objective of the project has been (and will be) to identify locations and sources of fecal contamination that negatively impact Saginaw Bay, especially at sites that the public uses for recreational purposes**

# Source tracking

## Can we determine where the contamination originates?



*Bacteriodes*

We can exploit subtle differences in gene sequences of bacteria that grow in varying hosts. Here, we use primers designed for the 16S rDNA from *Bacteroides* from host organisms.

```
                900                                930
 rural:    gcaacgcgcagaaccttaccagcccttgannatgtcnangaccggtcgcagagatgtgactc
L11663:    gcaacgcgcagaaccttaccagcccttgacatgtccantaccggtcgcagagatgtgactc
L11662:    gcaacgcgcagaaccttaccagcccttgacatgtccantaccggtcgcagagatgtgactc
L11661:    gcaacgcgcagaaccttaccagcccttgacatgtccantaccggtcgcagagatgtgactc
                     970
 rural:    ttcggagcntngagcacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggtt
L11663:    ttcggagcatggagcacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggtt
L11662:    ttcggagcatggagcacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggtt
L11661:    ttcggagcatggagcacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggtt
                                        1052
 rural:    aagtcccgcaacgagcgcaaccccctcnctcttagttgctaccatttagttgagcactctaagg
L11663:    aagtcccgcaacgagcgcaaccccctc-cttagttgctaccatttagttgagcactctaagg
L11662:    aagtcccgcaacgagcgcaaccccctc-cttagttgctaccatttagttgagcactctaagg
L11661:    aagtcccgcaacgagcgcaaccccctc-cttagttgctaccatttagttgagcactctaagg
                                                  1140
 rural:    agactgccggtgataagccgcgaggaaggtggggatgacgtcaagtcctcatg-cgcttacg
L11663:    agactgccggtgataagccgcgaggaaggtggggatgacgtcaagtcctcatggcccttacg
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L11661:    agactgccggtgataagccgcgaggaaggtggggatgacgtcaagtcctcatggcccttacg
                                       1170
 rural:     ggctgggctacacacgtgctac
L11663:     ggctgggctacacacgtgctac
L11662:     ggctgn-ctacacacgtgctac
L11661:     ggctgggctacacacgtgctac
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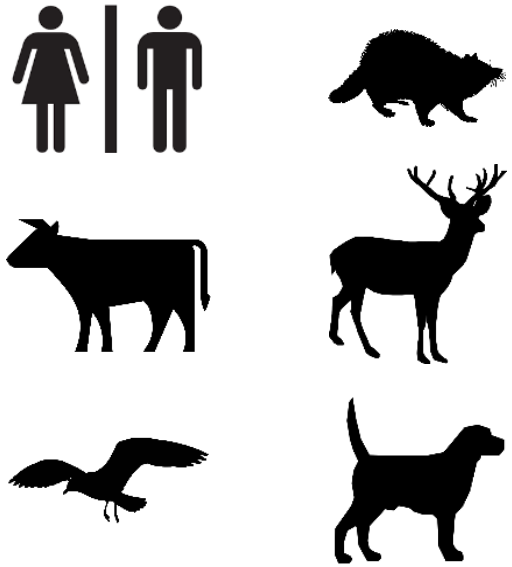
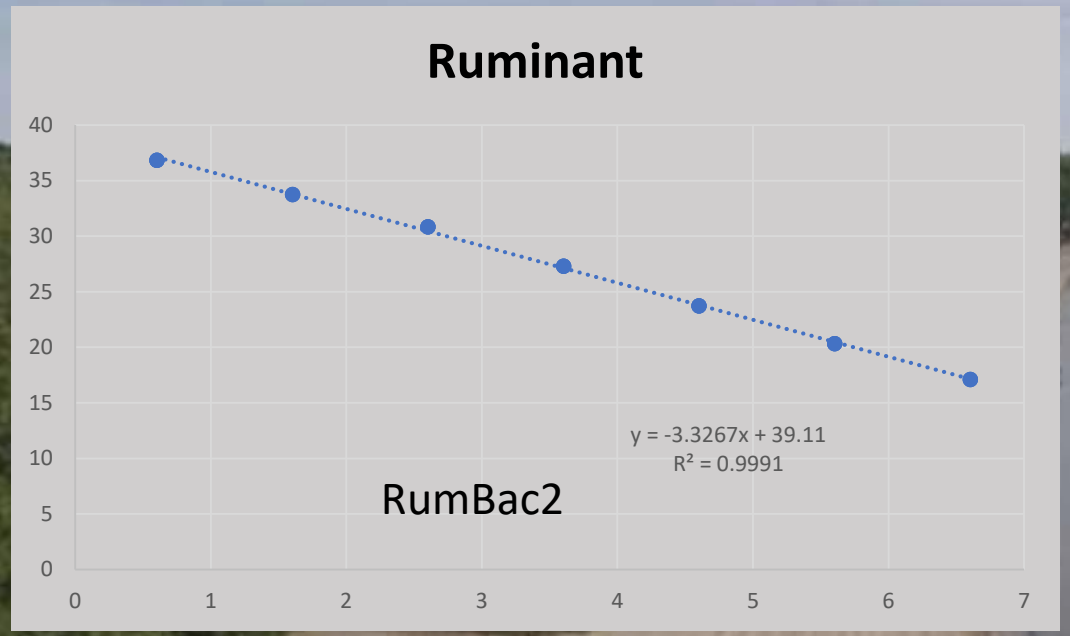
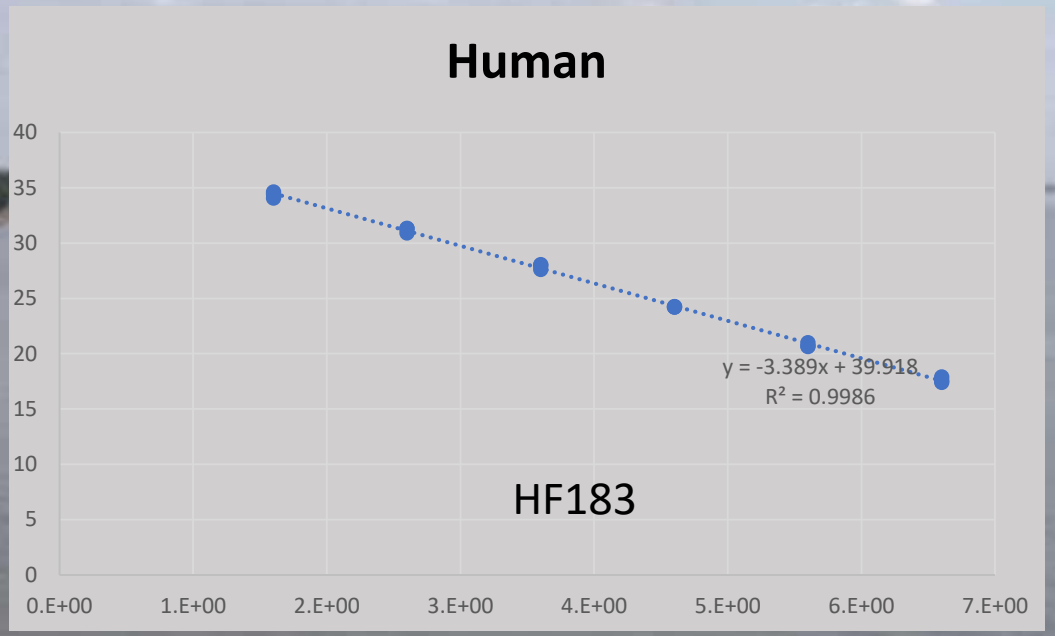
www.biotechniques.org

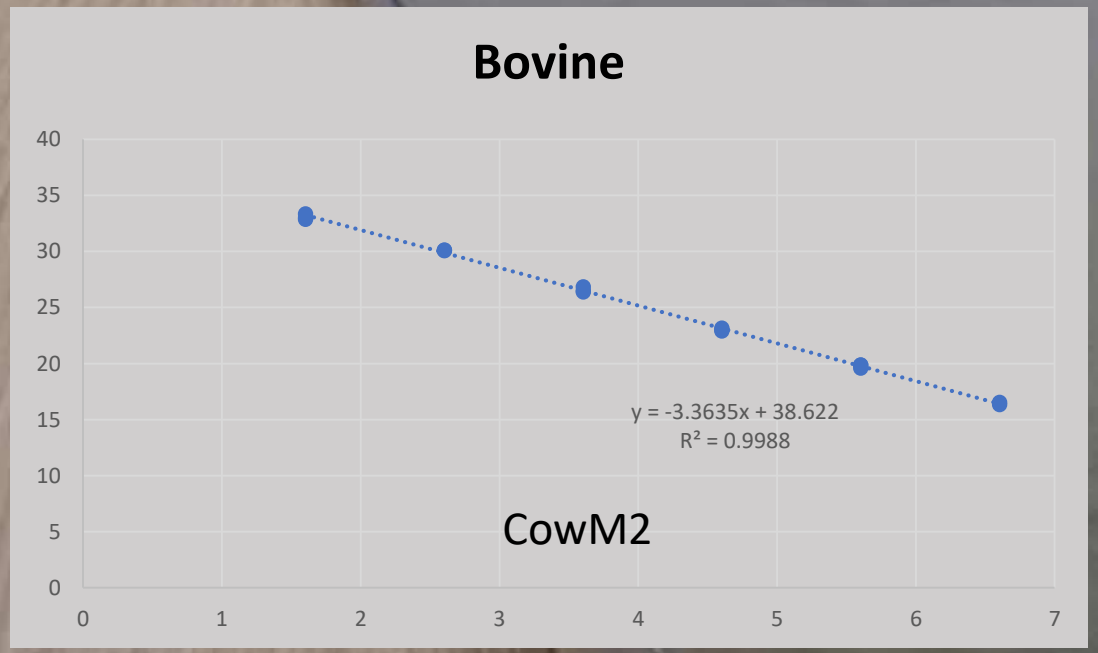
**Identify source(s)**



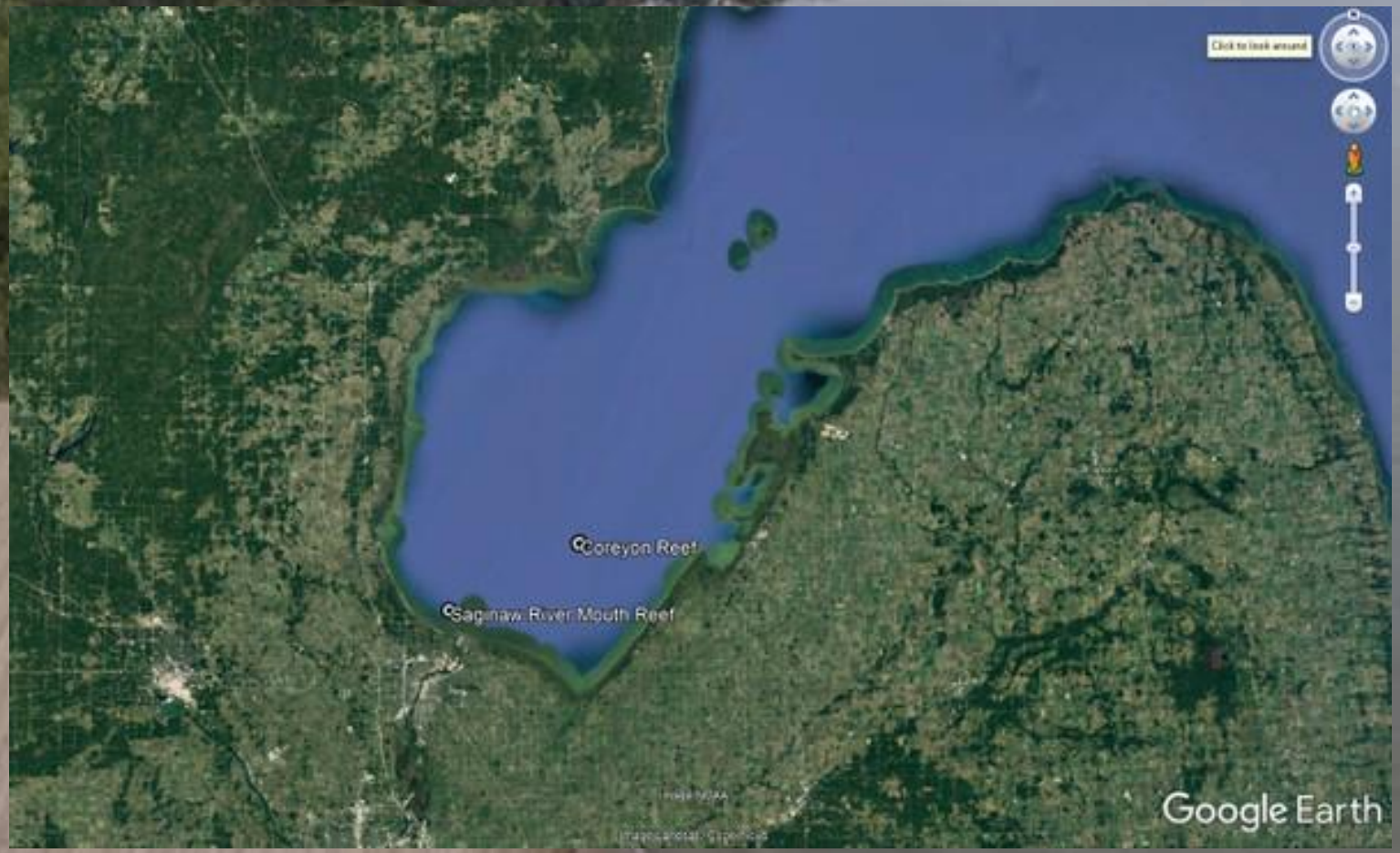
# qPCR standard curves for each marker



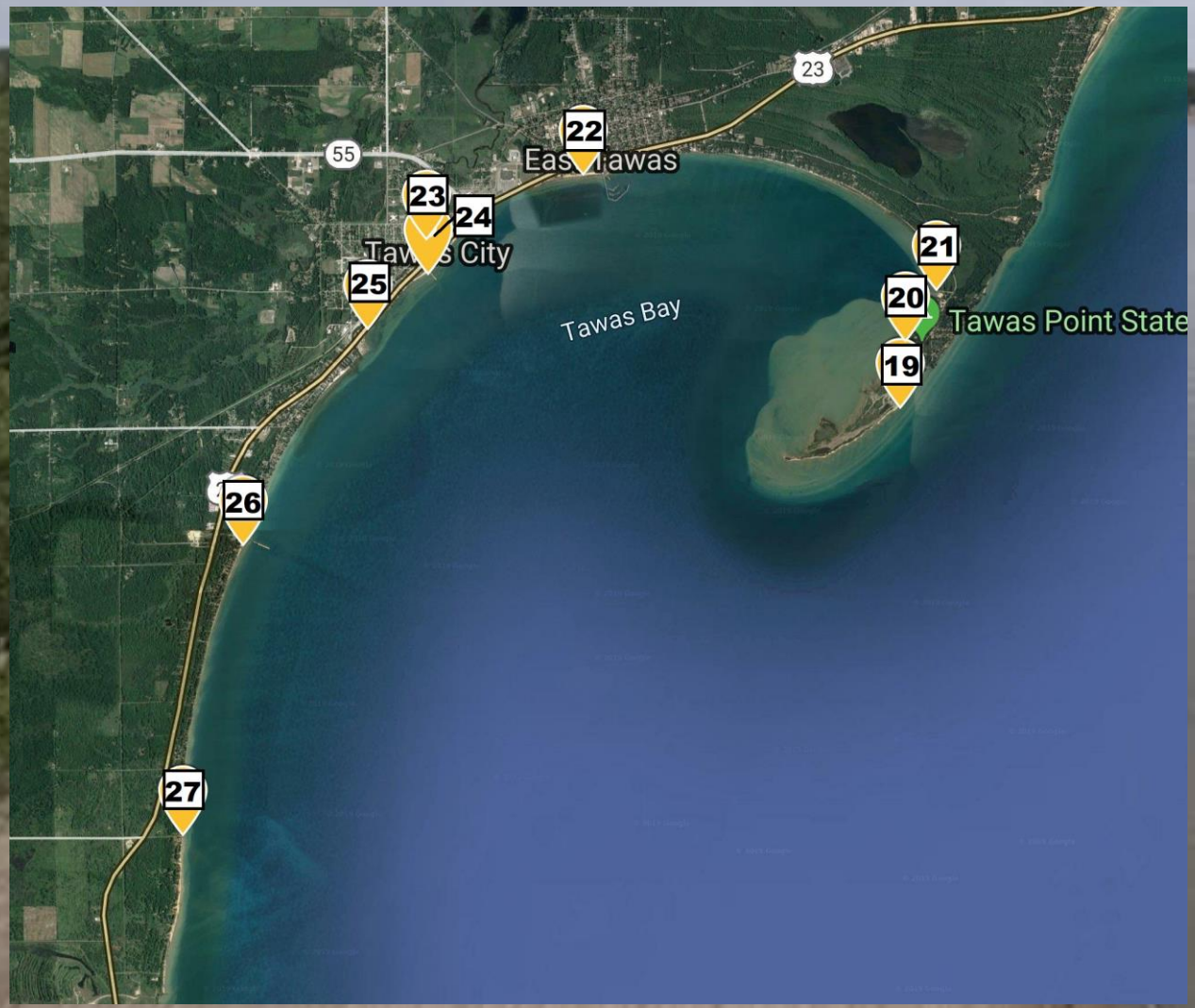



# Our home:  the Saginaw Bay Watershed

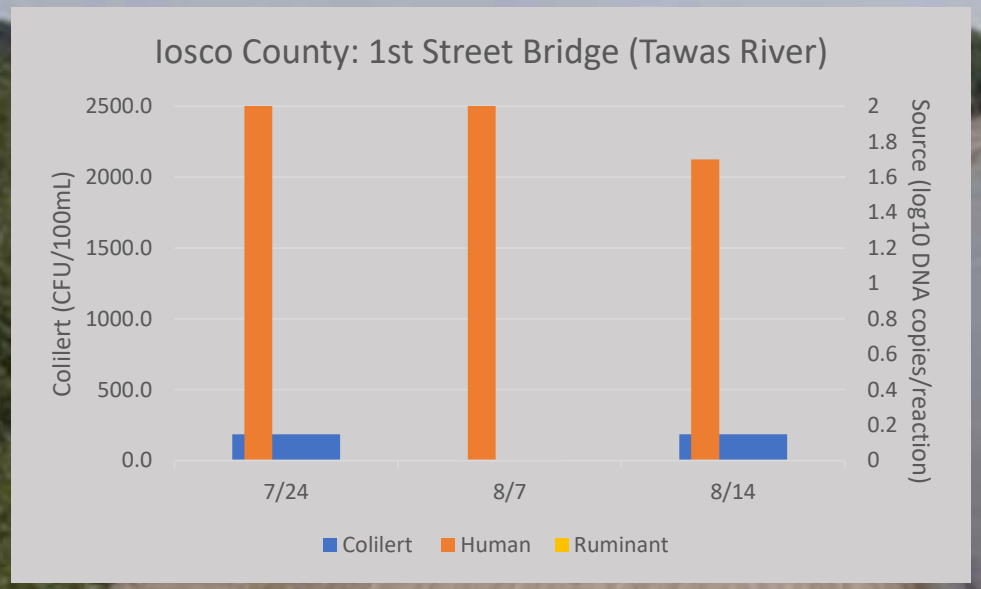


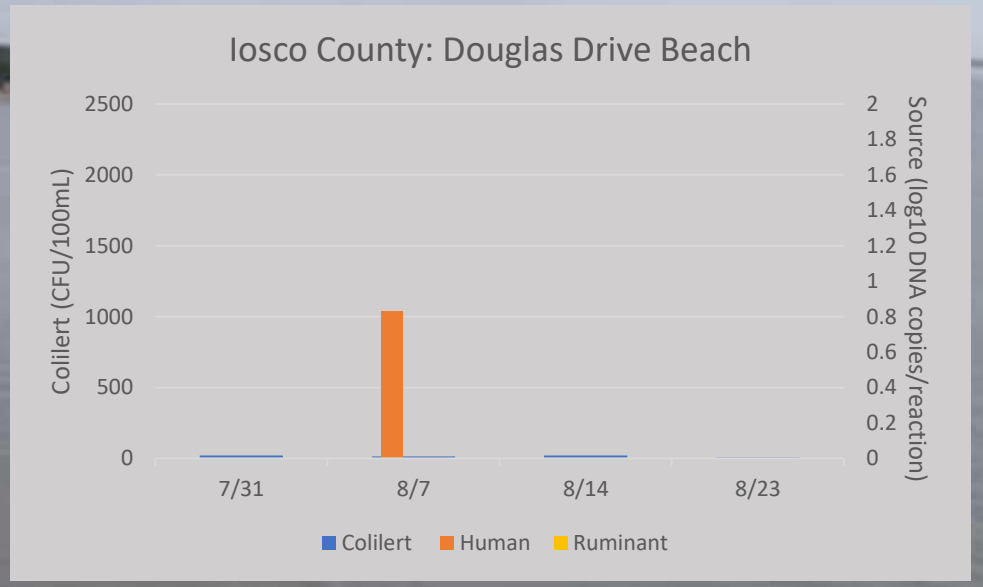
# Iosco County source tracking sampling sites 2018

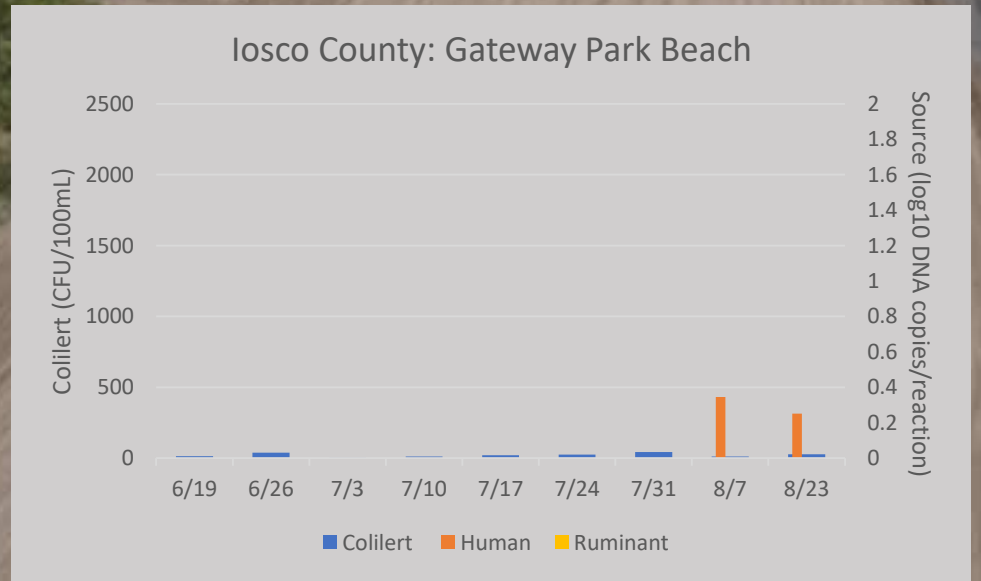


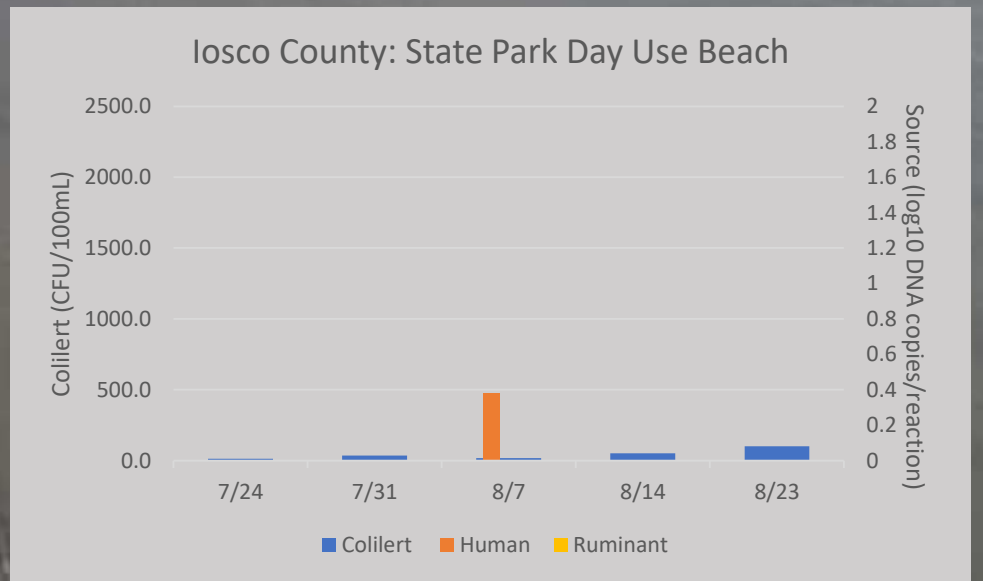

| Site # | Site Name |
|---|---|
| 19 | Tawas State Park - Day Use |
| 20 | Tawas State Park - Campground |
| 21 | County Road |
| 22 | East Tawas |
| 23 | 1st Street |
| 24 | Tawas City |
| 25 | Gateway |
| 26 | Douglas Drive |
| 27 | Alabaster |

# Example of pilot study results

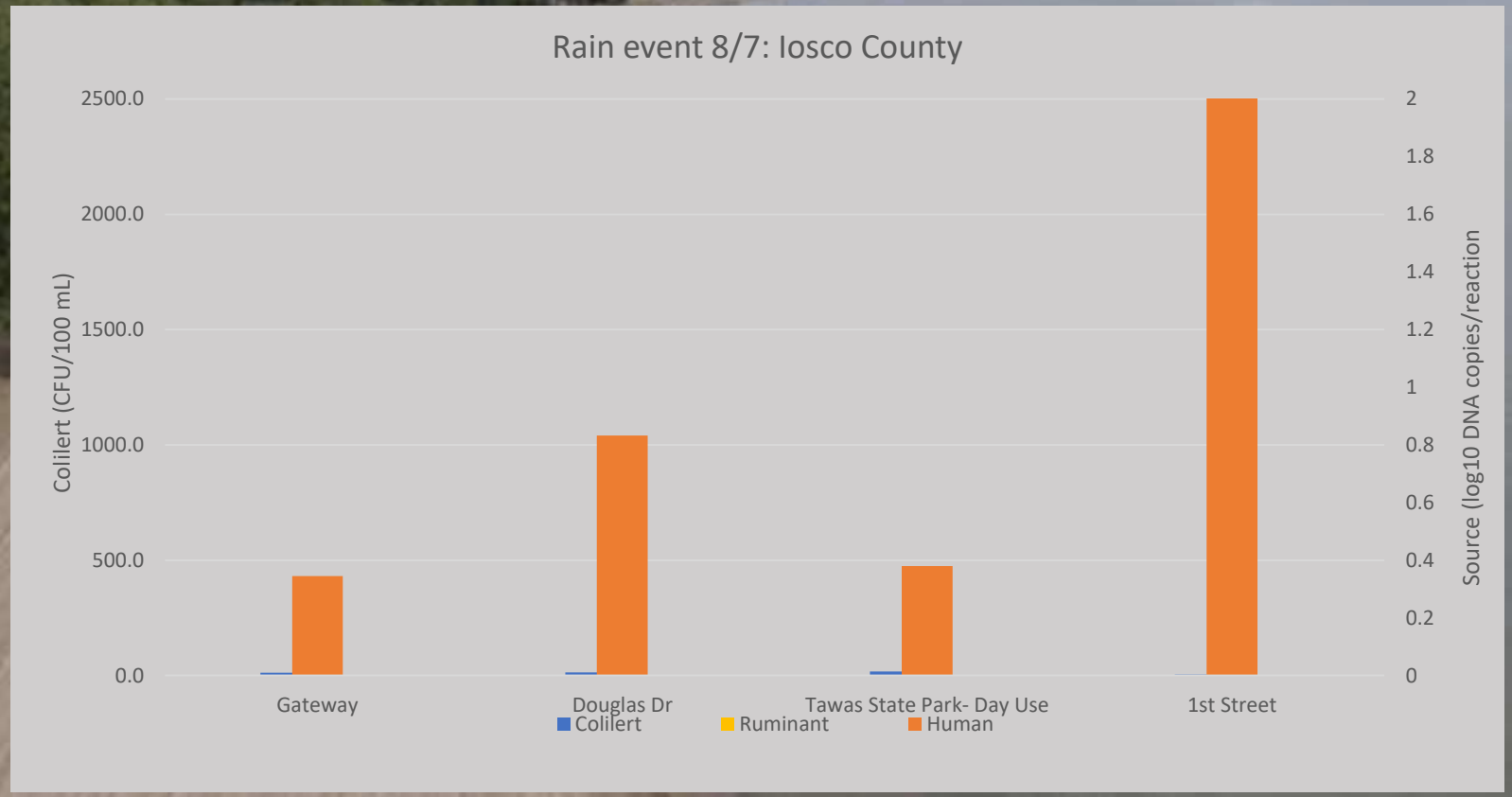


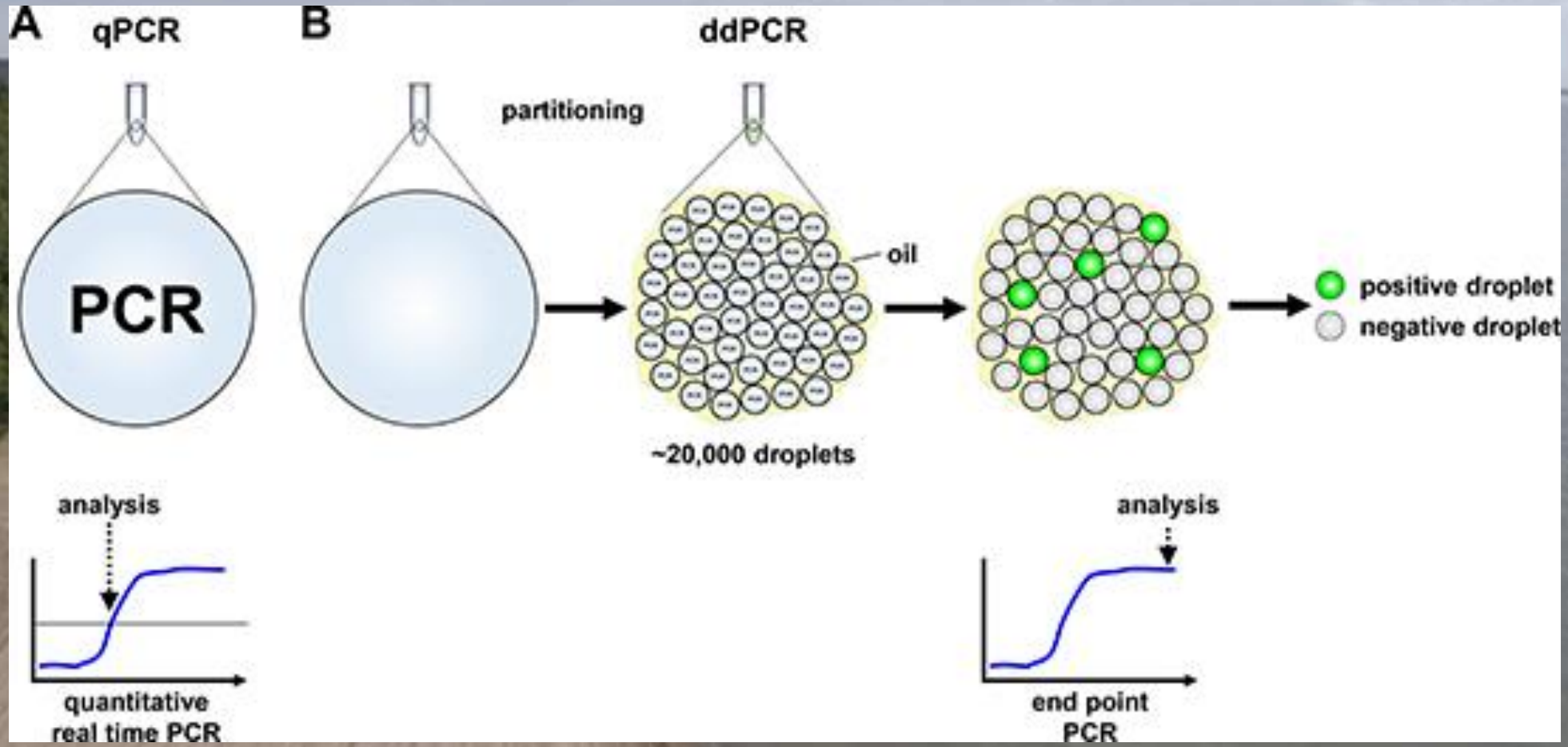
# Starting to piece things together…



# Bio-Rad digital droplet PCR system


Photo provided by Matt Flood, MSU

# ddPCR




Oncotarget. 2017; 8:85234-85251.

# What now???

Filters have been stored for source tracking analysis from beaches and inlets for the entire season of 2019

New markers!

Continue optimization of ddPCR methods

Consider upstream sites for analysis

# Acknowledgements

My students: Olivia Bishop, Trenton Vogel, Emily Greeson, April Lukowski, Marc Dean
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EPA: Rich Haugland, Orin Shanks, Mano Sivaganesan, Kevin Oshima
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