



Characterization of Human Fecal Pollution in Water

February 2020



Overview

- New Parameter
- No Current Regulatory Criteria
 - No “126 geometric mean” reference
- It is up to RA staff to innovate real world use of data
- Overview Mission Reach Coordinated Monitoring Effort



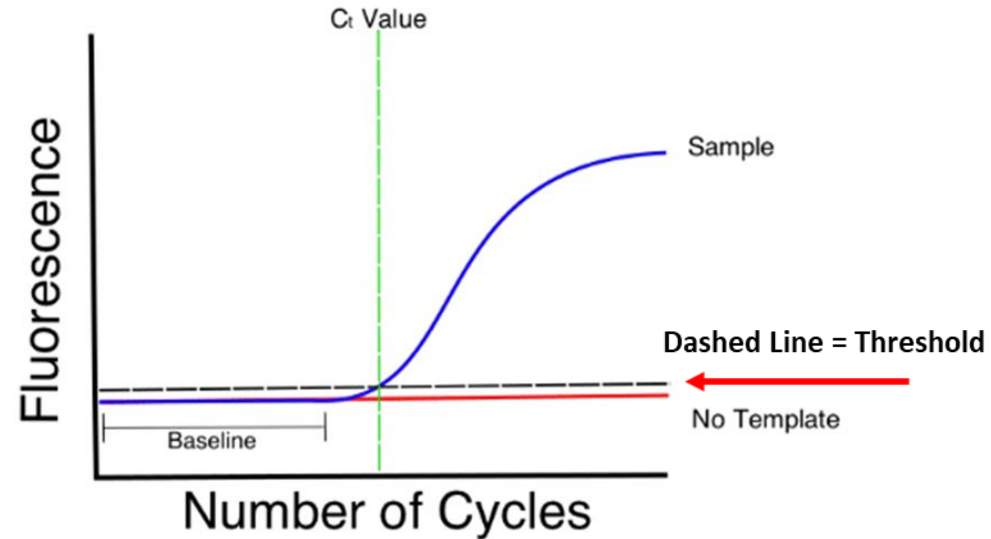
Discussion Coordination

- Definitions
- Updated Monitoring
- Current Progress
- Real World Tested Results
- Cost
- How Does SARA Utilize this Data?



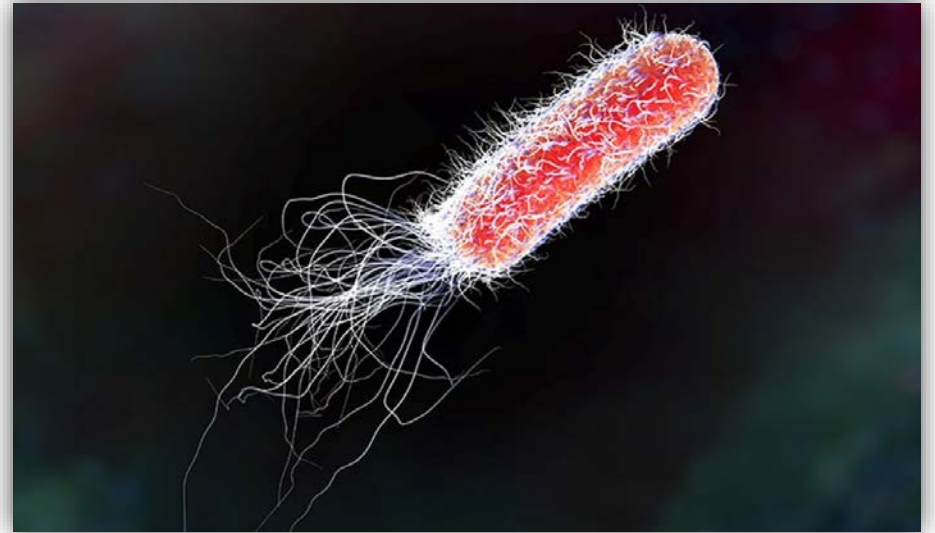
Definitions

- *Bacteroides* - Human-associated gene sequence commonly found in human feces and/or secondary human fecal pollution
- Threshold - The point at which a reaction reaches a fluorescent intensity above background levels
- Ct Value - The cycle number at which the sample reaction curve intersects the threshold line
 - This value tells how many cycles it took to detect a real signal from the sample



Updated Monitoring

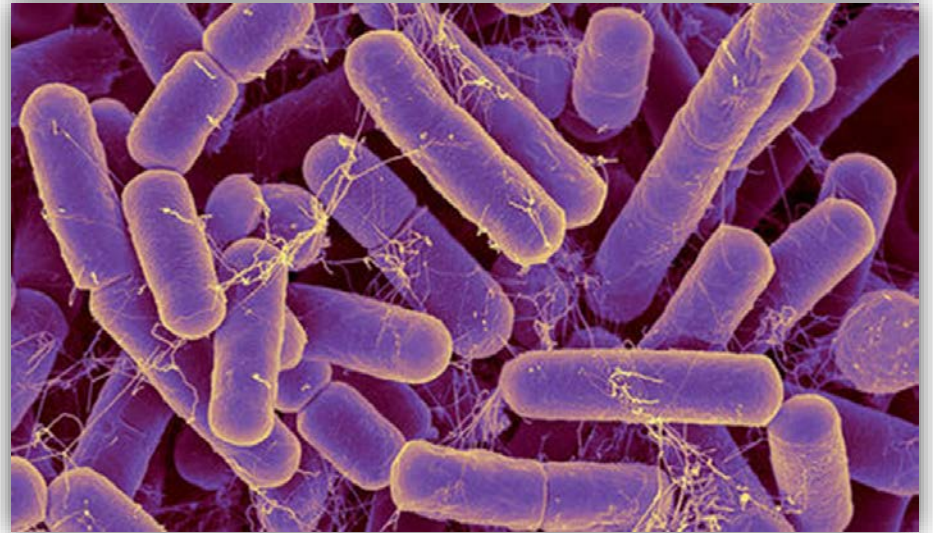
- *E. coli*
- Currently approved statewide fecal pollution indicator
 - Why we use it:
 - Can be easily quantified
 - Quick turn around (18-hour incubation)
 - Cheap (~\$10 of supplies)
 - Why it's problematic:
 - *E. coli* can come from any source, species, or location
 - Current method (IDEXX) does not discriminate species
 - *E. coli* presence does not clearly identify human influence



Updated Monitoring

- *Bacteroides*
- The presence of Bacteroides in environmental waters is generally considered an indicator of human* fecal pollution (EPA 1696)

E. coli* ≠ *Bacteroides



Current Progress

- Hired Molecular Biologist, Mike Martinez, 8/26/2019
- Cross-training completed 9/25/2019
- Qualitative* HF183 (Presence/Absence) SOP updated and effective 11/13/2019
- Quantitative HF183 SOP/research start date 10/7/2019
 - Dr. Orin Shanks (EPA)
 - Dr. Vikram Kapoor (UTSA)
 - Dr. Terry Gentry (TAMU)
- Principles of Quantitative PCR and Data Analysis training 12/10/2019-12/13/2019
- Parameter online effective 05/2020
- Sampling approved on Fee Schedule effective 07/01/2020



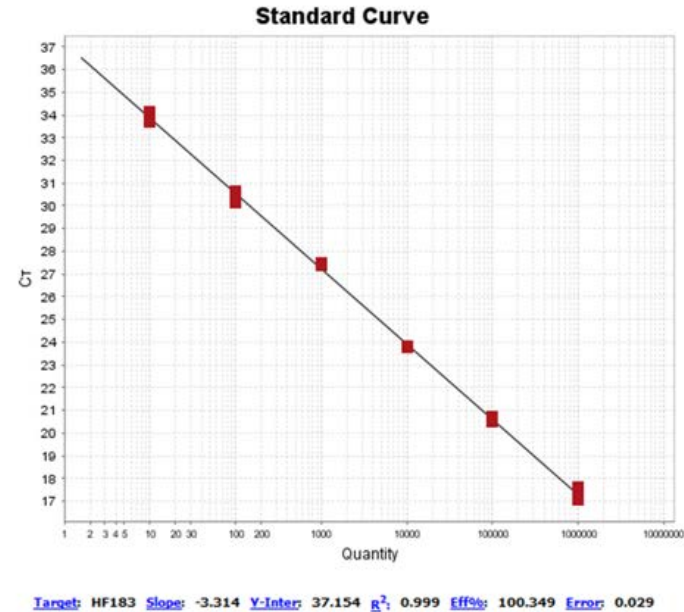
Current Progress

- How are we establishing confidence?
 - Employing method specific quality control procedures
- How are we going above and beyond?
 - Positive control (SARA WWTP influent) to ensure amplification and compare Ct values
 - Performing analysis at a range above method limits to reduce need for dilution and reanalysis



Current Progress

- What are the results?
 - Reported as Gene Marker Copies/100 mL
 - Commonly referred to simply as “copies”



Real World Tested Results

Positive Control – Salitrillo WWTP

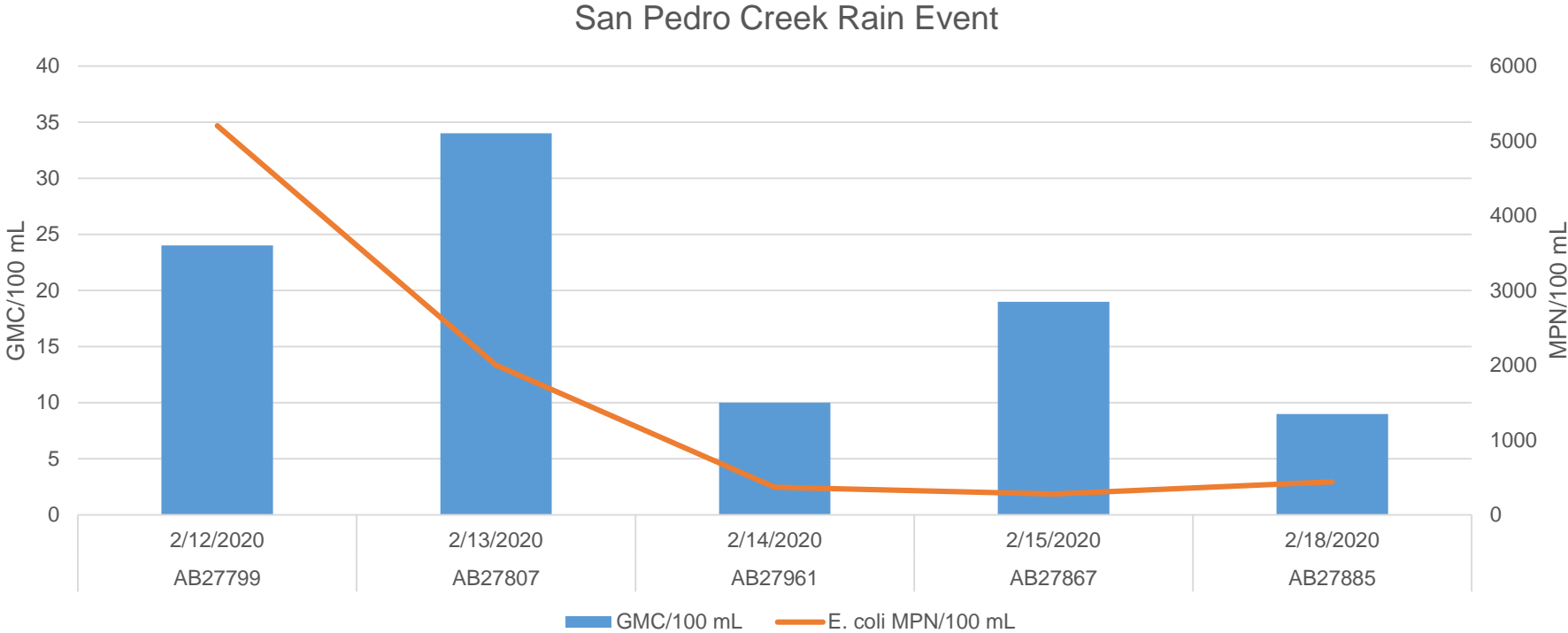
- January 22, 2020 1020
- Estimated GMC
 - 126,055 GMC/100 mL

San Pedro Creek (SARA_S0069)

- January 17, 2020 0640
- E. coli result
 - 13,000 MPN/100 mL
- BST HF-183 Result
 - Present for HF-183
- Estimated Gene Marker Copy/100 mL
 - 23 GMC/100 mL



Real World Tested Results



Real World Tested Results

- Storm Drain no. 9 downstream of Augusta St.
 - January 8, 2020 1235
 - E. coli result
 - >24,000 MPN/100 mL
 - BST HF-183 Result
 - Present for HF-183
 - Estimated Gene Marker Copy/100 mL
 - 250,776 GMC/100 mL



Cost

Internal Support (equipment only)

- Cost to run 1 Sample = **\$181.65**
 - 18 Standard Curve Wells - **\$56**
 - 3 Method Blanks (9 Wells, 3 Filters, 3 Extractions) - **\$61**
 - 3 No Template Control Wells - **\$9.33**
 - 6 Positive Control Wells – **\$18.66**
 - 1 Sample (6 Wells, 1 Filter, 1 Extraction) – **\$29.66**
 - 1 PCR Plate - **\$7**
 - Each subsequent sample will cost only \$29.66 because the same set of controls are used for analysis

External Clients (equipment+time)

- Molecular Biologist labor cost
 - 7-hour manual processing - **\$161.52**
- Data Review
 - 1.5 hours lab validation - **\$52.00**
 - 0.5 hours Quality Assurance validation - **\$30.00**
- **Roughly \$425* per sample**
- Sample result deliverable in 3-5 business days



How Does SARA Utilize this Data?

- Public
 - Swimming
 - Green/Red Flag
 - There is not a standard criteria for “healthy waters”
 - Must establish a history of *Bacteroides* quantities
 - Planning study alongside Mission Reach pilot
- Pollution Investigation
 - Correlation to positive control (degree of contamination)



Recommendation

- Currently proposed Mission Reach Daily *E. coli* Monitoring pilot effort
 - In addition to daily *E. coli* monitoring:
 - Collect BST at all sites first day of monitoring
 - Collect BST weekly from lowest sampling site on the reach
 - Collect BST quarterly at all sites
- Selection of additional monitoring sites to research BST
- Dilution of positive control study



Questions?

