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Office of Research and Development

Free Water Research Webinar Series

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

June 27, 2018

TODAY'S TOPIC:

Use of Microbial Source Tracking (MST) Tools in Waterborne Disease Outbreak Response

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Webinar Summary

Fecal pollution in recreational and drinking source waters can result in outbreaks leading to the transmission of disease. Information on the sources of fecal pollution is important because the level of human health risk can change from one pollution source to another. Understanding the source of disease causing enteric pathogens (e.g., norovirus) in outbreak environments is vital for determining and prioritizing remediation strategies. General fecal indicators, such as E. coli and enterococci, are typically used to assess fecal pollution; however, these methodologies do not discriminate between pollution sources. Recent advancements in the field of molecular biology have led to the development of microbial source tracking (MST) tools that can characterize fecal pollution from different animal groups.

The Centers for Disease Control and Prevention (CDC) is utilizing MST tools developed by U.S. Environmental Protection Agency (EPA) scientists for environmental investigations of waterborne outbreaks. For this webinar EPA will provide an overview the Agency's MST method development activities, and CDC will highlight a response to a recent waterborne outbreak where an EPA developed human-associated MST procedure was employed to help confirm a source of norovirus. This case scenario demonstrates how EPA and CDC interagency collaborations provide invaluable assistance to state environmental investigations of waterborne outbreaks.

Orin C. Shanks, Ph.D. (Contact: orin.shanks@epa.gov)

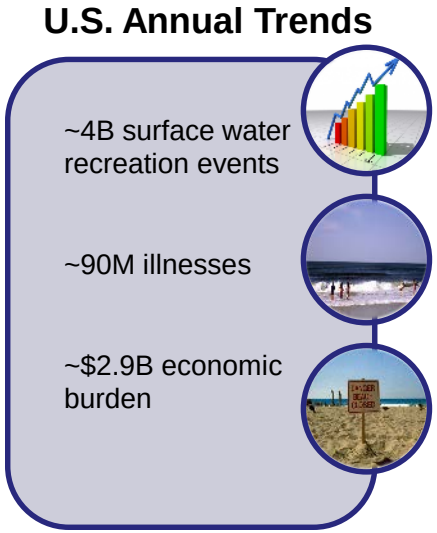
Dr. Shanks is a research geneticist with EPA's Office of Research and Development. His primary research area is the development, validation, and implementation of molecular technologies for environmental water quality management. Over the years, he has investigated the identification of host-associated genetic markers of fecal pollution, fate and transport of nucleic acids, utility of molecular methods for water quality monitoring, and has developed quantitative real-time polymerase chain reaction (PCR) methods. Dr. Shanks received his undergraduate and Master's degrees from the University of Wyoming and his Ph.D. from Oregon State University.

Presentation Overview

1. Microbial Source Tracking Overview
2. EPA Method Implementation Activities
3. CDC Waterborne Disease Outbreak Response
4. Case Study: CDC Response using Microbial Source Tracking

Fecal Pollution is a Nationwide Problem

- Fecal microbes are a common biological contaminant in U.S. surface waters.
- Public health, economic, and ecological impacts



DeFlorio-Barker et al. (2018) Environmental Health 17:3

EPA Responsibilities

Protect and Restore Waters for Recreational Use

- Clean Water Act 1972

Risk Assessment of Beach Contaminants

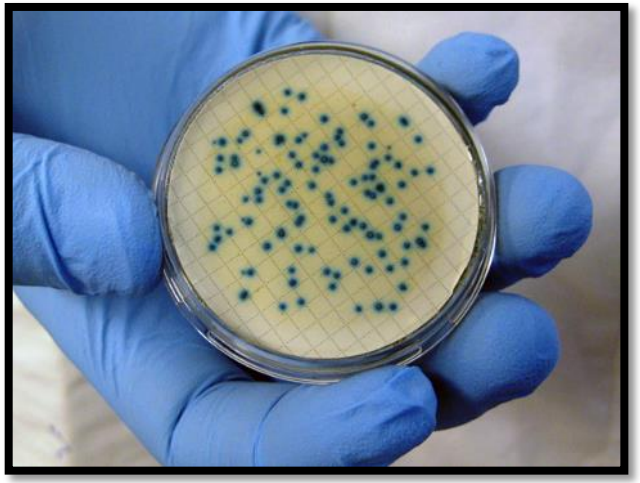
- BEACH Act 2000
- Recommendation of Ambient Water Quality Criteria

Management of Point and Non-Point Pollution Sources

- Total Maximum Daily Load programs
- National Pollutant Discharge Elimination System programs
- Combined Sewer Overflow consent decrees

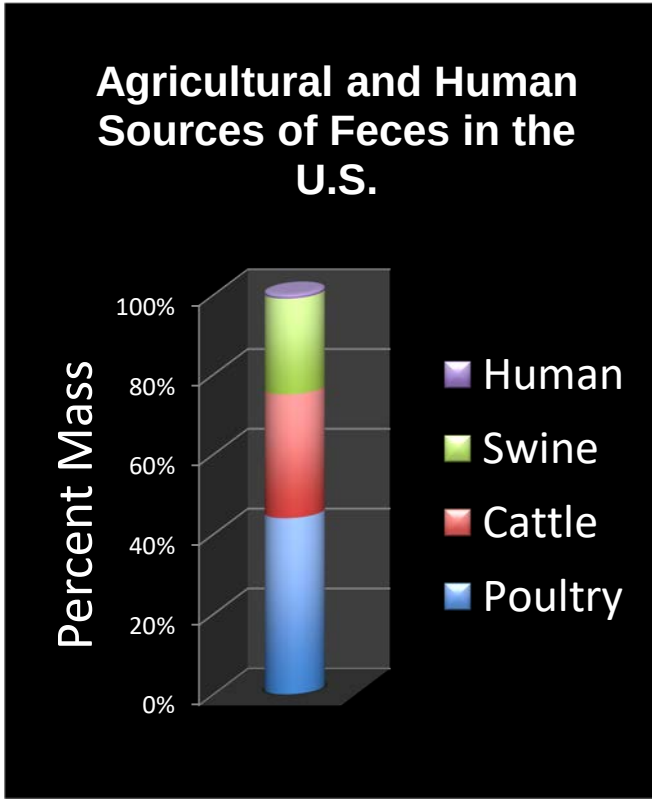
Current Fecal Pollution Management Tools

- Based on general fecal indicators
- Measure of total fecal pollution
- Presence in water is a warning signal of public health risk
- Do not discriminate between sources



Source of Fecal Pollution is Important

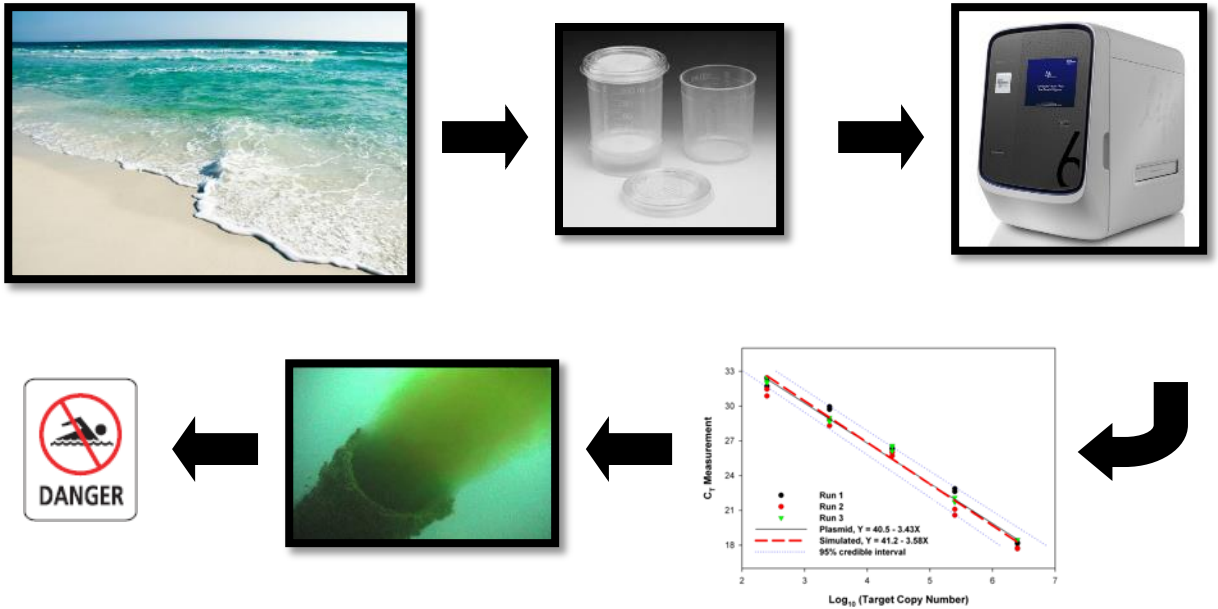
- Estimated $1x10^9$ tons of fecal material produced in U.S. each year.
  - Human (0.01%)
- Public health risk can vary by source.
- Mitigation strategies can vary by source.



Estimated $1x10^9$ tons of fecal material produced in U.S. each year (human, ~0.01%). RL *Kellogg, CH Lander, DC Moffitt, N Gollehon - NRCS and ERS GSA Publ. No. NPS00-0579. Washington, DC: USDA, 2000*

The Microbial Source Tracking Concept

SOLUTION … Method designed to collect, isolate, identify, and measure a **host-associated identifier** from an environmental sample.



Many Potential Applications

- Total Maximum Daily Load support tool
- Impaired site prioritization for remediation
- Evaluation of a best management practices
- Stormwater discharge management support
- Combined sewer overflow monitoring
- Waterborne disease outbreak response investigative tool

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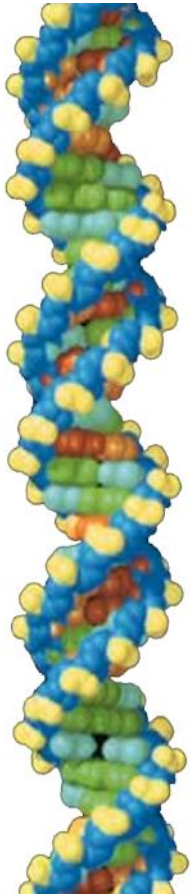


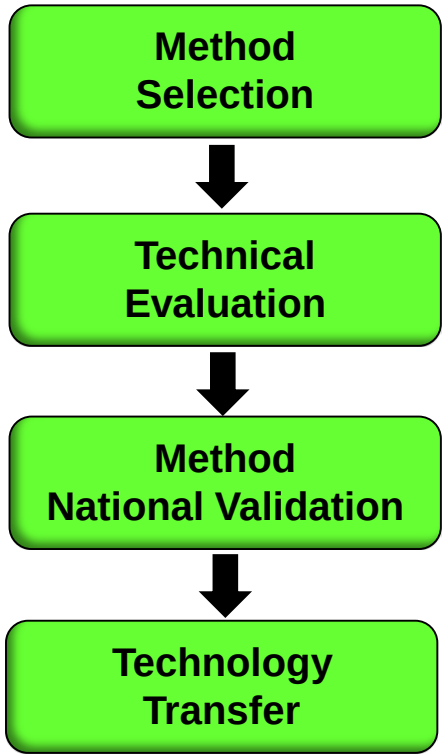
National Implementation: Status

- No standardized method recognized by federal agency
- Public interest rapidly growing
- Intensively studied in scientific arena
- California Microbial Source Identification Manual (Technical Report #804)

Microbial Source Tracking Tool Implementation Wish List

Goal	Description
Clear Host Association	Strong evidence of close link with a specific pollution source
Quantitative Metric	Identifier concentrations are accurate and reproducible
Expert Consensus	Agreement among majority of experts
Standardization	Complete standard operating procedure
National Validation	Multiple laboratory confirmation that the method adequately meets application needs
Technology Transfer Kit	Application guidance, training tools, easy to use kit, and reference materials

Human Microbial Source Tracking Method Implementation Strategy



- Expert consensus

- Expert review
- Method optimization

- Establish Standard Operating Procedure (SOP)
- Conduct national multiple laboratory study

- EPA Method publication
- Reference material development
- Tool kit

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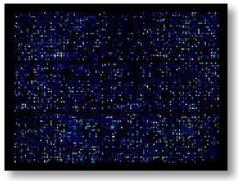
Many Microbial Source Tracking Methods Available

- Microarray
- Next generation sequencing
- End-point PCR
- Quantitative real-time PCR
- Digital PCR
- Immuno-magnetic separation
- Terminal restriction fragment length polymorphism
- Selective bacterial culturing
- Antibiotic resistance profiling
- Chemical detection
- Canine scent detection



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Human Method Selection by Expert Consensus

- Source Identification Protocol Project
  - 5 organizations formed technical lead team
  - Public challenge via blinded study
  - 27 expert laboratories
  - 41 methods

- Majority of experts (>90%) favor a **PCR-based technology.**

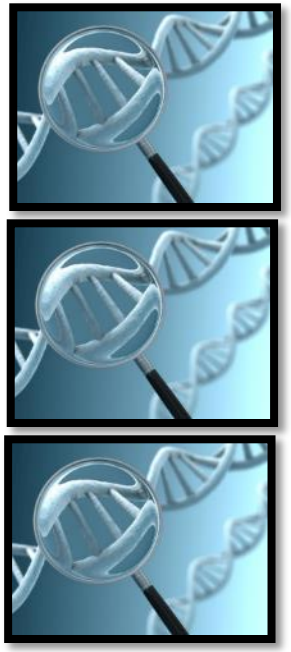
- PCR-based methods are highly reproducible across labs only when protocols are **standardized.**

- Identification of top human-associated method
  HF183 qPCR

Boehm *et al.* (2013) Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research* 47: 6812-6828.
Ebentier *et al.* (2013) Evaluation of the repeatability and reproducibility of a suite of PCR-based microbial source tracking methods. *Water Research* 47: 6839-6848.
Layton *et al.* (2013) Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Research* 47: 6897-6908.
Stewart *et al.* (2013) Recommendations following a multi-laboratory comparison of MST methods. *Water Research* 47: 6829-6838.

Technical Evaluation

HF183 qPCR Technical Evaluation via Peer-Review



- Administered by team of experts
  - Government and academic sectors
- Rigorous laboratory assessment subject to peer-review
- Protocol adherence to Minimum Information for Publication of qPCR Experiments (MIQE)

Bustin, S. A. et al. (2009). The MIQE Guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*. *55*: 611-622.

- Optimization to reagents custom designed for environmental samples

Green, H. C. *et al.* (2014). Improved HF183 Quantitative Real-Time PCR Assay for Characterization of Human Fecal Pollution in Ambient Surface Water Samples. *Applied and Environmental Microbiology*. 80: 3086-3094.

Method National Validation

HF183 qPCR EPA Method National Validation - Overview

- Formal study conducted by EPA
  - Office of Water
  - Office of Research & Development
- HF183 qPCR
- 14 Laboratory Participants
  - Fresh and marine water matrices
- Supplied with
  - Standard protocols
  - Reference DNA materials
  - Sewage spike material
  - Blinded filter set (n = 18)
  - All reagents and consumables

Method National Validation

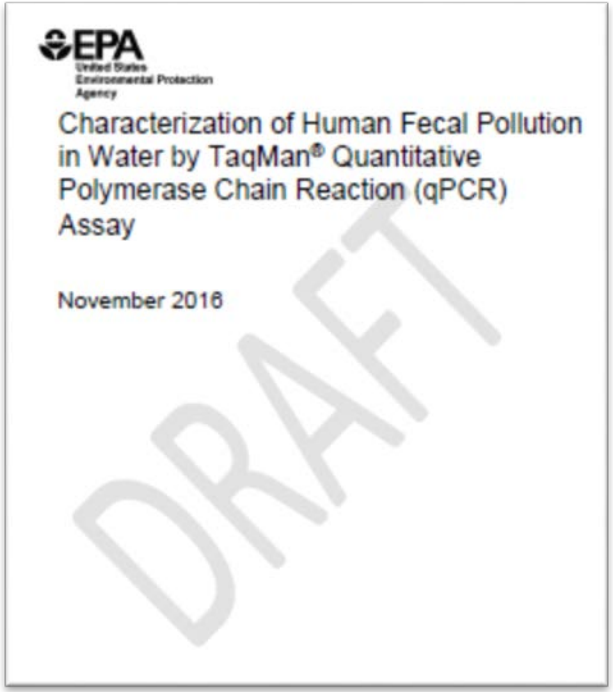
Customized HF183 qPCR Data Acceptance Metrics

Type	Metric
Calibration Curve Model	R^2
	Amplification efficiency (E)
Extraneous DNA	No-template controls (NTC)
	Method extraction blank (MEB)
Matrix and Amplification Control Proficiency	Internal amplification control proficiency
	Sample processing control proficiency
Test Sample	Inhibition screen with IAC
	Matrix interference with SPC
	Lower limit of quantification (LLOQ)

Shanks *et al.* (2016) Data Acceptance Criteria for Standardized Human-Associated Fecal Source Identification Quantitative Real-Time PCR Methods. *Applied and Environmental Microbiology* 82: 2773-2782.

Technology Transfer

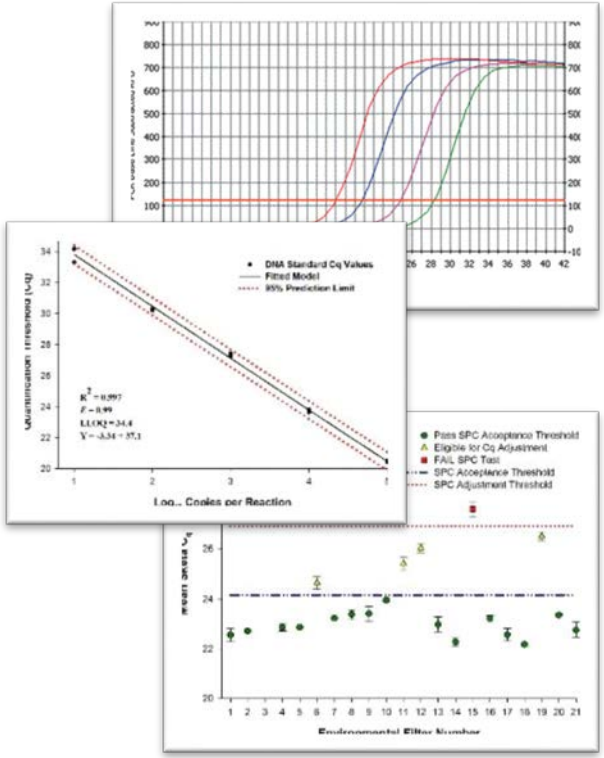
Draft HF183 qPCR EPA Method Content Overview



- Safety
- Laboratory organization
- Equipment, reagents, and supplies
- Sample collection, handling and storage
- Standardized laboratory procedures
- Quality controls
- Data analysis and calculations

Technology Transfer

HF183 qPCR Automated Data Analysis Tool



- Simplify complex calculations
- Ensure standardized analysis
- Implement data acceptance metrics
- Concentration estimates with error

-Microsoft Excel Workbook
-Standardized data input
-Summary report

Technology Transfer

Self-Administered HF183 qPCR Method Proficiency Test

- Successfully complete
  - Prior to environmental sample testing
  - After new reference material preparations
- Six metrics based on
  - National laboratory validation
  - Reagent manufacturer recommendations
  - qPCR experts
- Training and management tool

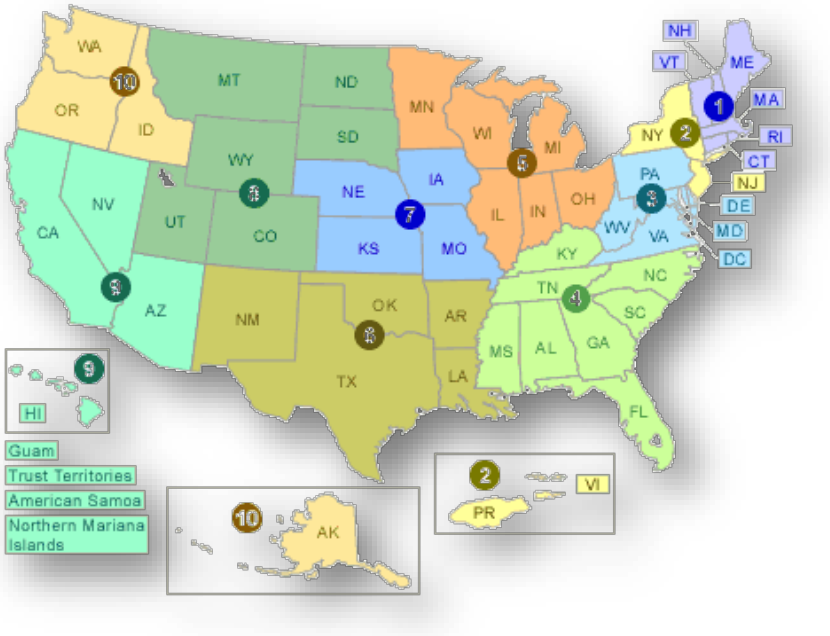
Technology Transfer

HF183 qPCR Standardized Reference DNA Material Development

- National implementation requires high quality reference DNA material.
- Centralized and standardized source
- Not feasible for EPA to manufacture and distribute
- Interagency Agreement with National Institute of Standards and Technology

Technology Transfer

HF183 qPCR Technology Outreach Activities



- Technical support network
- Communication tools
- Training opportunities
- Application guides
- Cooperative partnerships
  - States, tribes, and other local labs
  - Association of Pubic Health Laboratories MOU
  - Federal agencies

Mia Mattioli, Ph.D. (Contact: kuk9@cdc.gov)

Dr. Mattioli is an environmental engineer with CDC's Environmental Microbiology Laboratory of the Waterborne Disease Prevention Branch within the Division of Foodborne, Waterborne and Environmental Diseases and the National Center for Emerging and Zoonotic Infectious Diseases. She serves as the lead for domestic projects, and her research is focused on the intersection between the environment and human health with a specific interest in the relationship between, and fate and transport of, fecal indicators and enteric pathogens in environmental matrices. Dr. Mattioli leads the environmental investigations of waterborne outbreak responses by the CDC. She has a Bachelor of Science in Biological Engineering from the University of Georgia and a Master and Ph.D. in Environmental Engineering from Stanford University.

The findings and conclusions in this presentation are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Waterborne Outbreaks

- 2 or more persons epidemiologically linked after exposure to same water source
- **Illness types:** depends on etiological agent but can include gastrointestinal, respiratory, and/or skin infections
- **Water types**: drinking, recreational, industrial, healthcare, and agricultural water use

- **2013-2014 reported U.S. outbreaks**
  - **Drinking water** – 42 outbreaks, 1,006 illnesses, **13 deaths**
  - **Recreational water** – **85 outbreaks, 3,543 illnesses**, 4 deaths
  - **Environmental** – 15 outbreaks, 226 illnesses, 9 deaths
  - **Undetermined** – 12 outbreaks, 63 illnesses, and 3 deaths

Outbreaks: 154

Illnesses: 4,838

Deaths: 20

CDC Outbreak Response

- Outbreak investigations by CDC result from requests for assistance from state and local health departments and abroad.
- Requests include assistance with
  - Responding to emergencies.
  - Quantifying impact of diseases.
  - Investigating infectious disease outbreaks.
- Epi-Aids and Lab-Aids are short-term requests for on site CDC assistance.

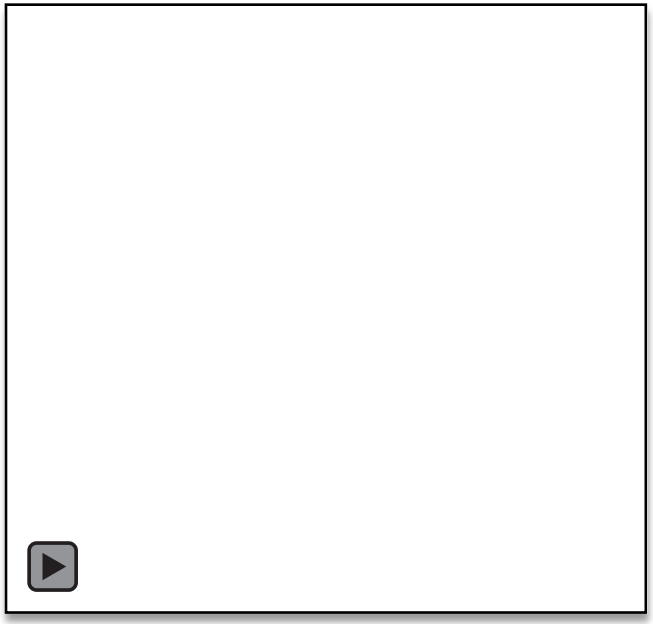
2017 CDC Hurricane Response in Puerto Rico

WDPB Environmental Microbiology Lab

- **Laboratory research**
  - Develop **methods for recovering** low concentration microbes from environment
  - Investigate pathogen **prevalence, ecology, and risk factors** associated with waterborne disease
  - Understand **transport, survival, and disinfection susceptibility** of microbes in environment
- **Outbreak and emergency response**
  - Investigate **the cause and source** of waterborne disease and outbreaks
  - Conduct sampling to link suspected **etiologic agents** between case and water exposure
  - Assay for **water quality** parameters, **microbial indicators**, and **fecal source markers**

WDPB Environmental Microbiology Lab

- **WDPB EM labs focuses on the environment**
  - Drinking water
  - Surface water (rivers, lakes, storm water runoff)
  - Recreational water (swimming pools, spas, lakes)
  - Wastewater, reclaimed water, and gray water
  - Filter media and backflush (e.g., carbon and sand filters)
  - Soil, sediment, and biosolids
  - “Other”
- **Environments addressed by other CDC labs**
  - Surface sampling and survival on surfaces
  - Air sampling and aerosol biology
  - Biofilm analysis in manufactured systems (e.g., distribution systems, premise plumbing)



Ameba in collected water sample

Environmental Investigations in Waterborne Outbreaks

- Complement epi data suggesting water/environmental exposure route
- Link water samples and ill persons to confirm water as transmission vehicle
- Design environmental mitigation and remediation strategies
- **CDC EM lab actively involved in >110 outbreak responses domestically and abroad over last 10 years.**

2018 Atlanta Water Main Break

WDPB Outbreak Response Pathogens & Sources

Waterborne pathogens

- *Cryptosporidium*
- *Salmonella*
- *Escherichia coli* - shiga toxin producing
- Norovirus
- *Shigella*
- *Giardia*
- *Legionella*
- Hepatitis A and E
- *Campylobacter*
- *Naegleria fowleri*
- *Cyclospora*
- *Acanthamoeba*
- *Elizabethkingia*

Pathogen sources in outbreaks

- Human: feces, sewage, septage
- Mammals: dogs, deer, rat, hedgehogs, mouse, beaver
- Birds: goose, gull, ducks
- Livestock: cow, goat, llama, chickens, pigs
- Reptiles: turtles, frog, geckos, dragons
- Naturally present

WDPB Outbreak Response Environmental Sampling

Water

- **Grab sample** of 100 mL – 1 L for general fecal indicators (e.g., *E. coli*), physical/chemical water quality
- **Large-volume** via ultrafiltration of 10 L – 100+ L for pathogens

Soil/sediment/biosolids "grab sample" into sterile container or bag

Surface swabs/wipes shower head, water tap aerator

Other filters, water meter, pipe, garden hose, "slip 'n' slide," nasal rinsing device, contact lens case - *Collection procedures vary and are often improvised.*

Pathogen Detection in Environment Not Always Possible

- Die-off/inactivation and dilution
- Uncertainty regarding where to sample due to spatial variability
- Pathogens present at orders of magnitude lower levels than normal gut microflora
- Difficult to culture isolates from background microorganism community
- Time delays between contamination and sample collection
- Often multiple potential pathogen sources

Microbial Source Tracking in Outbreak Response

Tool to generate investigative leads

- Understand potential enteric pathogen sources in outbreak environment
- Identify, prioritize, and determine effectiveness of prevention/remediation strategies
- Evaluate health risks when
  - Unable to detect pathogen in suspected exposure route.
  - Post outbreak long term monitoring after pathogen no longer detectable.

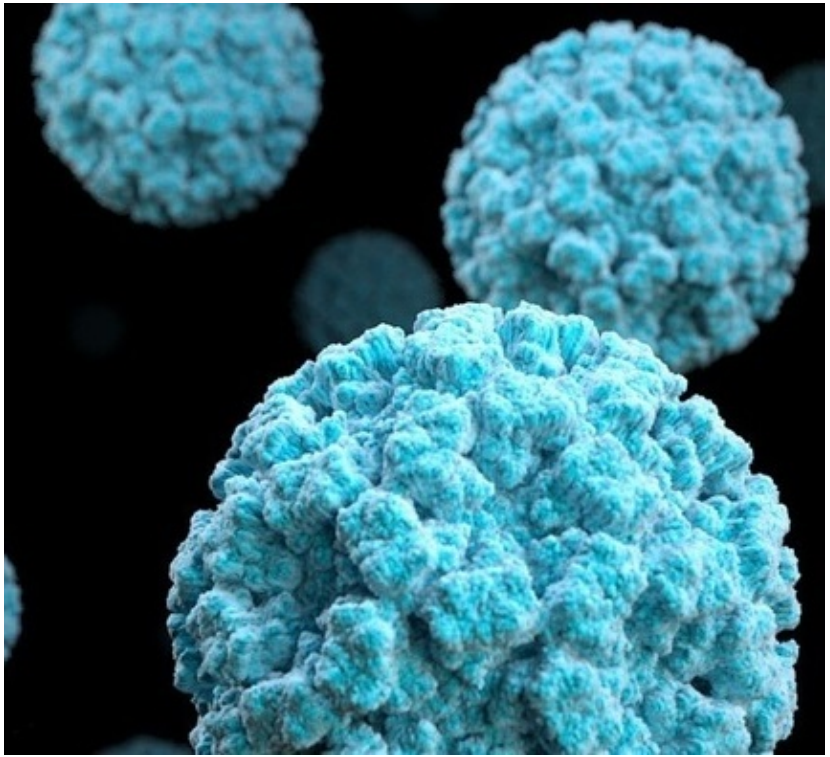
Goat feces sampling during *Crypto* outbreak

Norovirus in Drinking Water Well

MST to identify contamination link to water and evaluate health risk

Norovirus

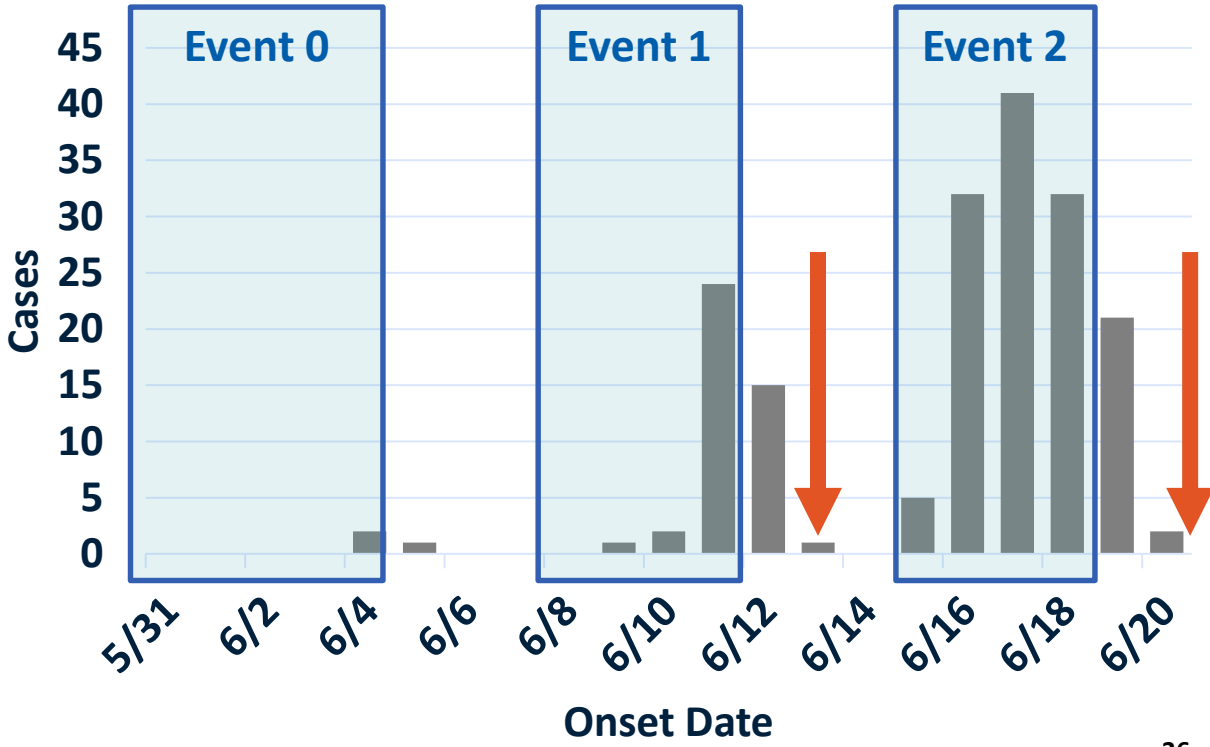
- Suspected cause of almost 20% of all diarrhea cases worldwide.
- Found in animals and humans but not considered zoonotic.
- Human norovirus consists of 3 genogroups: GI, **GII**, and GIV.
- Very contagious via infected person, food, water, or contaminated surfaces.
- Causes inflammation of stomach, intestines, or both leading to stomach pain, nausea, diarrhea, and vomiting.



Norovirus – Lopman et al., 2016 PLoS Medicine

Norovirus Outbreak Epidemiology (Case N = 179)

- Outbreak at camp reported June 13.
- Similar outbreak occurred at next event.
- Norovirus detect in 7 cases from **June 15.**
- **Well and creek water samples from June 21 GI *and* GII positive.**
- **Epi-/Lab-Aids** requested to prevent outbreak at large upcoming event (>3000 ppl).



Environmental Investigation Objectives

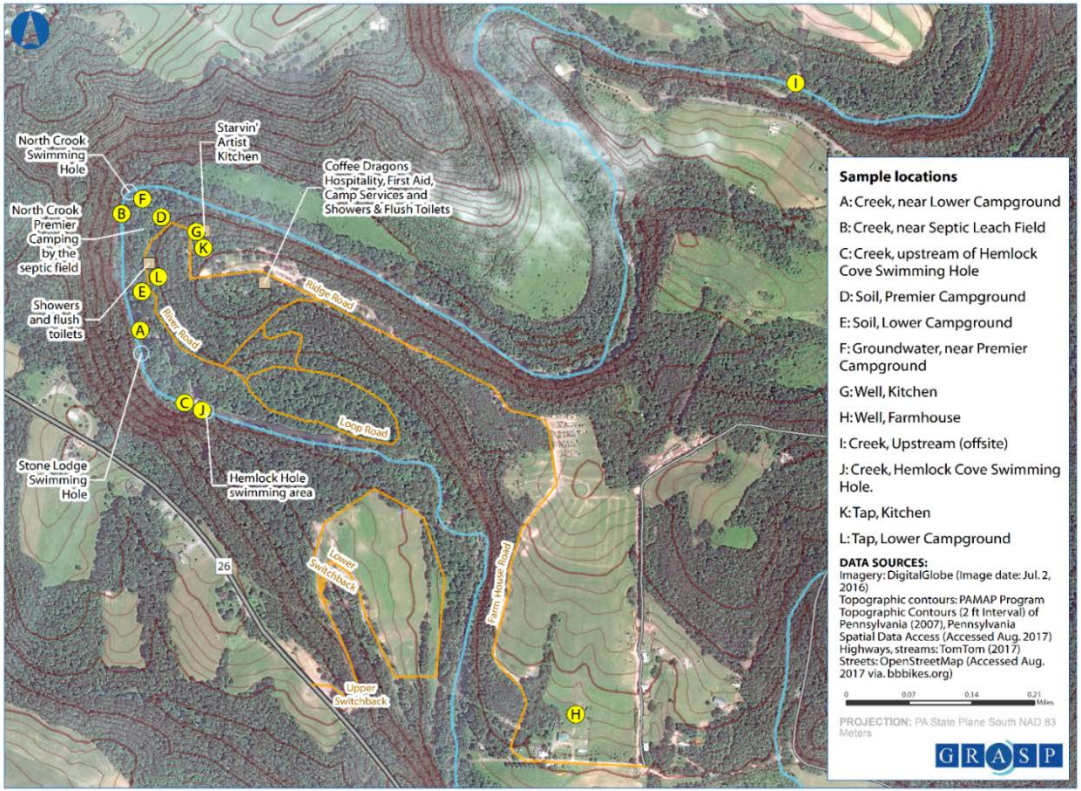
1. Determine outbreak pathogen presence in routes of water exposure at camp: drinking and creek water.

2. Evaluate whether camp's septic system was a source of fecal contamination to the creek and the camp well using EPA human source tracking marker.

3. Evaluate the health risks from exposure to recreational creek based on microbial contamination.

Recreational creek adjacent to camp grounds

Sampling Sites (N = 12)



- Drinking well, creek, and ground water sampled
  - Original drinking water well
  - Original creek sample site next to septic leach field
  - Upstream, adjacent to camp, and downstream creek
  - Kitchen tap post DIY disinfection system
  - Soil from campground above septic leach field
  - Newly install drinking well

Samples Collected

- Prior to CDC arrival, drinking water well taken offline, septic tank drained, and camp placed under boil water advisory.
- Ground water samples collected in collaboration with **EPA hydrogeologist** along ground water flow from septic leach field.
- Large volume ultrafiltration water
  - 335 L drinking well water
  - 200 L creek water
  - 40 L ground water
- Samples assayed for: norovirus GI/GII, adenovirus, enterovirus, *E. coli*, enterococci, F+ and somatic coliphage, **human-specific MST marker HF183**

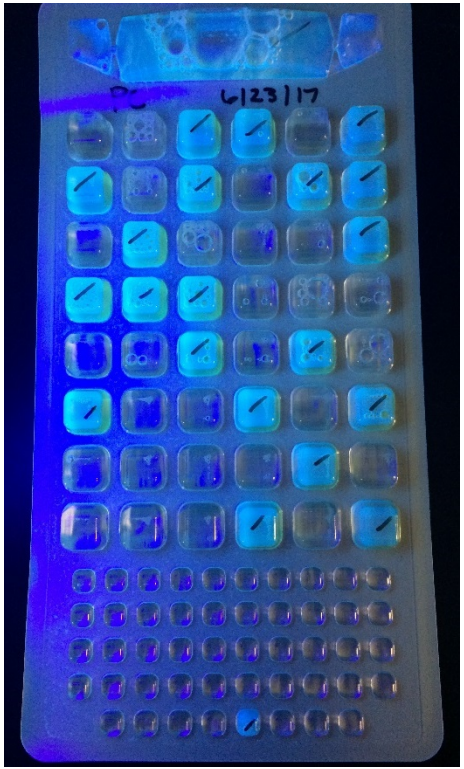
Pathogen Results

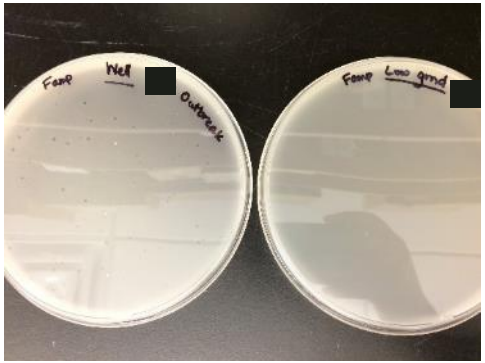
- Norovirus GI and GII detected in well again but not in creek
  - Typing matched clinical cases
- Adenovirus (general) in well, not creek
- Enterovirus in well, not creek
- No pathogens detected in ground water or soil

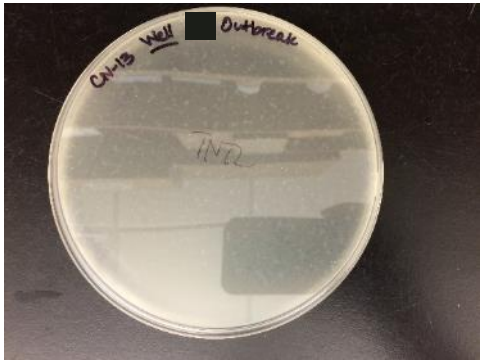
Toilet and showers connected to septic

General Fecal Indicator Results

- Drinking water well
  - Total coliforms: 223 MPN/100 ml
  - *E. coli*: 109 MPN/100 ml
  - Enterococci: 2.3 MPN/100 ml
  - F+ and somatic coliphage
    - Type G2 RNA F+ coliphage only
- Creek
  - *E. coli*: ≤ 35 MPN/100 ml by camp and 648 MPN/100 ml upstream
  - Enterococci: ≤ 100 MPN/100 ml
  - Somatic coliphage
- Ground water
  - *E. coli:* 100 MPN/100 ml
  - *Enterococci:* 148 MPN/100 ml
  - Somatic coliphage





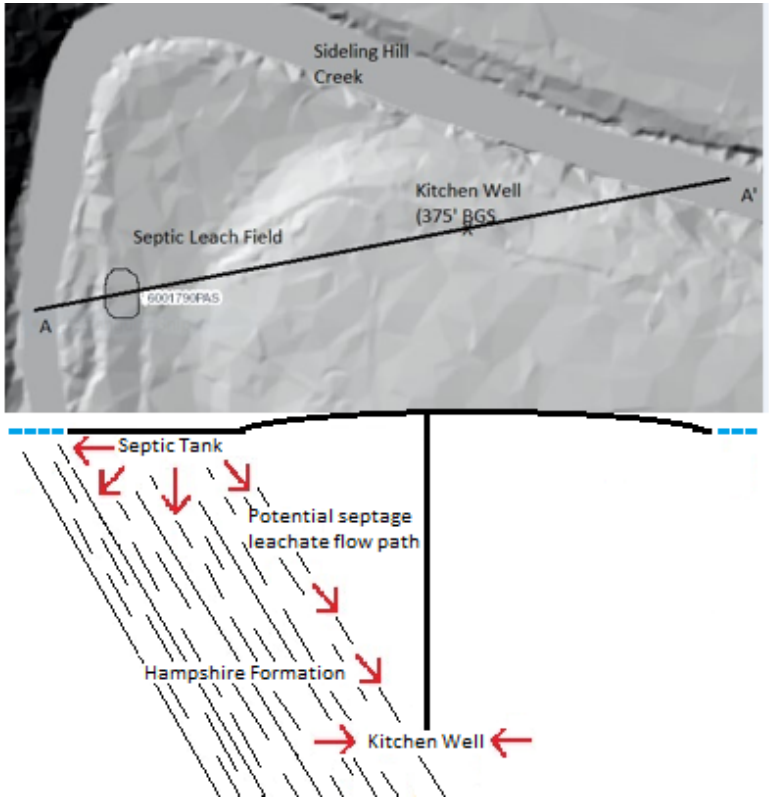


MST Results

- HF183 in drinking well water in June and July and in tap in June.
- HF183 detected in all creek samples *including upstream.*
- 1-$\log_{10}$ higher HF183 concentration in creek adjacent to camp translated to a **10-fold higher risk** from swimming by leach field. (Boehm et al., 2015 *ES&T Letters*)

MST Success

- Human MST marker results supported hydrogeological connection between septic system, drinking well, and recreational creek.
- General and pathogen-based fecal indicators used as microbial tracers of septic/human contamination.
- Health risks from rec water exposure estimated despite lack of pathogen detection **using HF183 marker.**



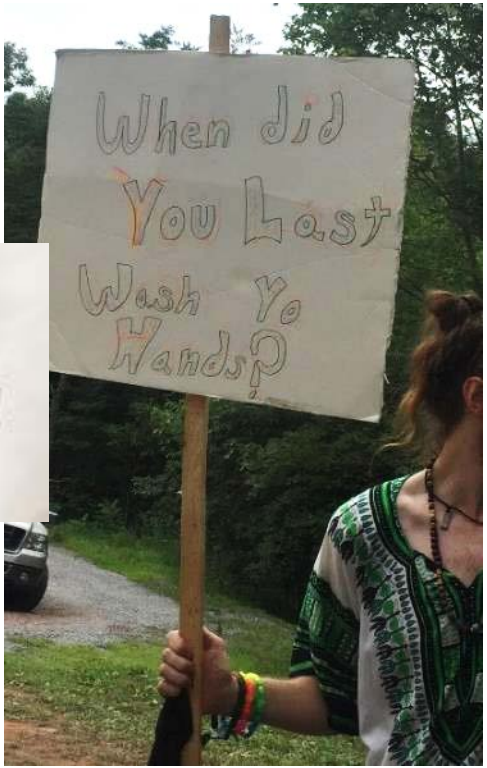
MST Limitations

- No standards for MST concentrations – how much is too much human fecal marker in rec water?
- Health risks based on concentration differences in *one time samples collected from a large, flowing environmental source.*
- No sample of the contamination source – assume HF183 high in septic.
- Difficult to interpret HF183 positive samples below rec water standards – differences in environmental decay?

Remediation and Prevention Strategies

New drinking water well

Temporary sanitation



Voluntary health promotion

Future of MST in Outbreak Response

- MST technologies proven to be promising investigative tool for CDC waterborne outbreak response.
- More work needed to understand the technology transfer requirements for state labs to use MST in response.
- More research needed to better understand associations between source tracking markers, pathogens, and health risk.
- **EPA and CDC priorities align in microbial source tracking.**

Questions & Answers Session