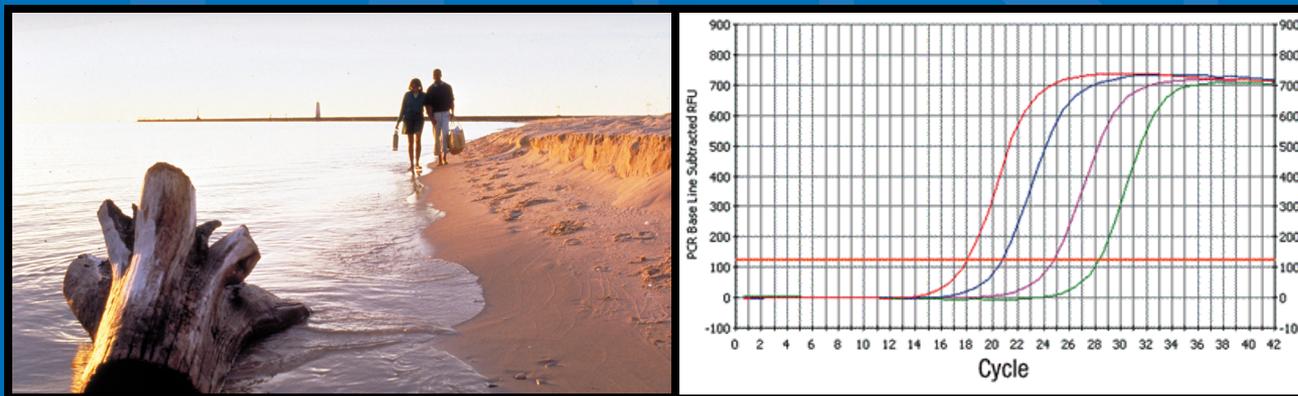


Microbial Source Tracking: From Basic Science to Management Tool

Orin C. Shanks



Presentation Overview

1. Overview
2. A Case Study
3. Some Observations



Disclaimer: *The views expressed in this presentation are those of the author[s] and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

Fecal Pollution is a Worldwide Problem

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

U.S. Annual Trends

~4B surface water recreation events

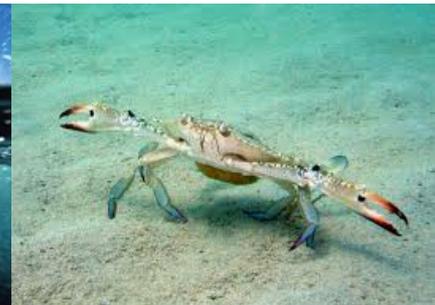
~90M illnesses

~\$2.9B economic burden

~\$10M for beach water programs



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)
- Development of new or revised ambient water quality criteria (AWQC)

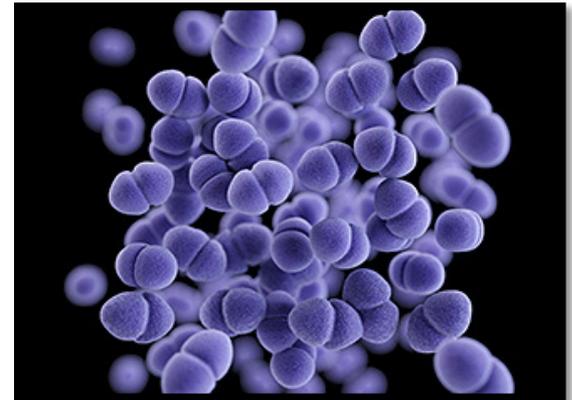
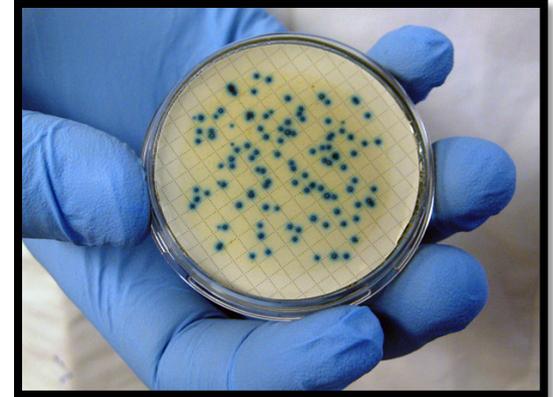
Management of Point and Non-Point Pollution Sources

- Total Maximum Daily Load (TMDL) programs
- National Pollutant Discharge Elimination System (NPDES) programs
- National Estuary Program (NEP)
- Combined Sewer Overflow (CSO) consent decrees



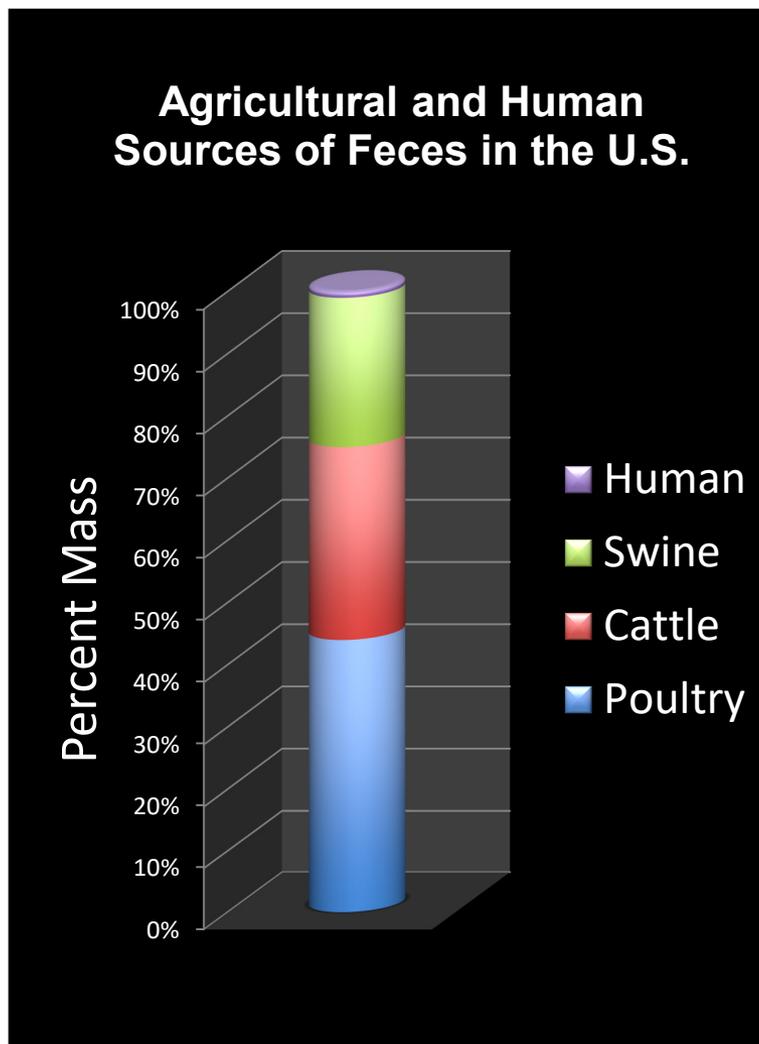
Current Fecal Pollution Management Tools

- General [fecal indicator bacteria](#)
- Widely distributed in most animals
- Presence in water is a warning signal of public health risk
- Used worldwide to manage fecal and sewage contamination



Source of Fecal Pollution is Important

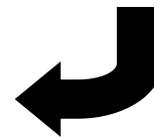
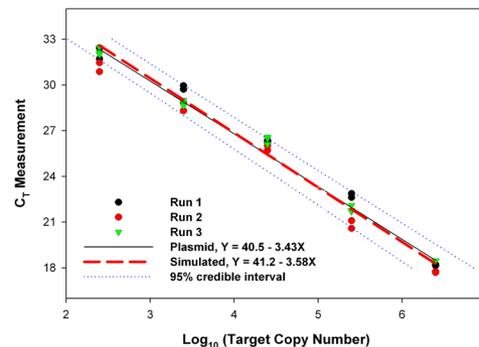
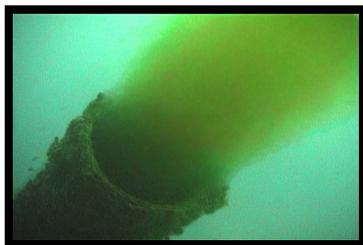
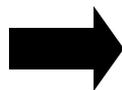
- Public health risk can vary by source
- Mitigation strategies can vary by source
- Source information improves water quality management and public safety



Estimated 1×10^9 tons of fecal material produced in U.S. each year (human, $\sim 0.01\%$). RL Kellogg, CH Lander, DC Moffitt, N Gollehon - NRCS and ERS GSA Publ. No. NPS00-0579. Washington, DC: USDA, 2000

A Microbial Source Tracking Solution

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



The Science Behind a Host-Associated Identifier

- **Gut Condition Differences**

- Diet
- Digestive physiology
- Temperature

- **Resource Competition**

- Space
- Nutrients



Many Applications for Water Quality Management

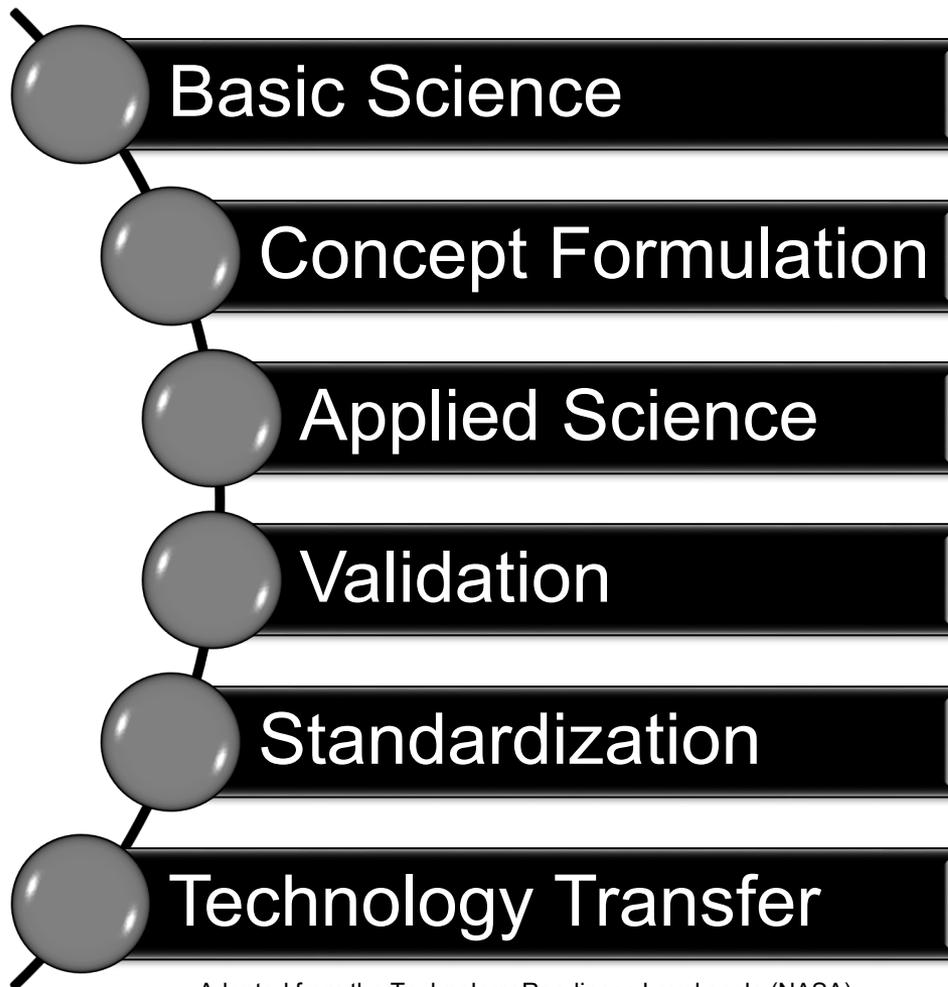
- Mixed use watershed management
- Impaired site prioritization for remediation
- Evaluation of a best management practices
- Nutrient discharge characterization
- Recreational water quality indicator
- Urban stormwater management tool
- Waterborne disease surveillance support
- Hazardous event response



Properties of an Ideal Microbial Source Tracking Management Tool

Goal	Description
Clear Host-Association	Strong evidence of close link with target pollution source
Known Host-Distribution	Broadly distributed across target population
Quantitative Metric	Absolute concentration information
Expert Consensus	Agreement among majority of experts
Standardization	Complete standard operating procedure available
Data Acceptance Metrics	Performance benchmarks to ensure high quality results
Validation	Multiple laboratory confirmation that the method adequately meets application needs
Field Demonstrations	Real-world examples with guidance for implementation
Technology Transfer Tools	Easy to use process, training opportunities, lab proficiency testing, troubleshooting tools, etc.

A Management Tool Development Map



Adapted from the Technology Readiness Level scale (NASA)

A Case Study: The HF183 Human Host-Identifier



- First reported in 2000 (Bernhard and Field 2000)
- Extensively studied:
 - Over 2,000+ citations
 - Wide range of applied science information:
 - Host distribution
 - Field applications (> 15 countries)
 - Fate and transport
 - Link to public health
- Strong track record in performance studies:
 - Top human method, 22 expert labs (Griffith et al. 2003)
 - Top human method, 27 expert labs (Boehm et al. 2013)

Is Cultured *Bacteroides* spp. an Ideal Fecal Indicator Bacteria?

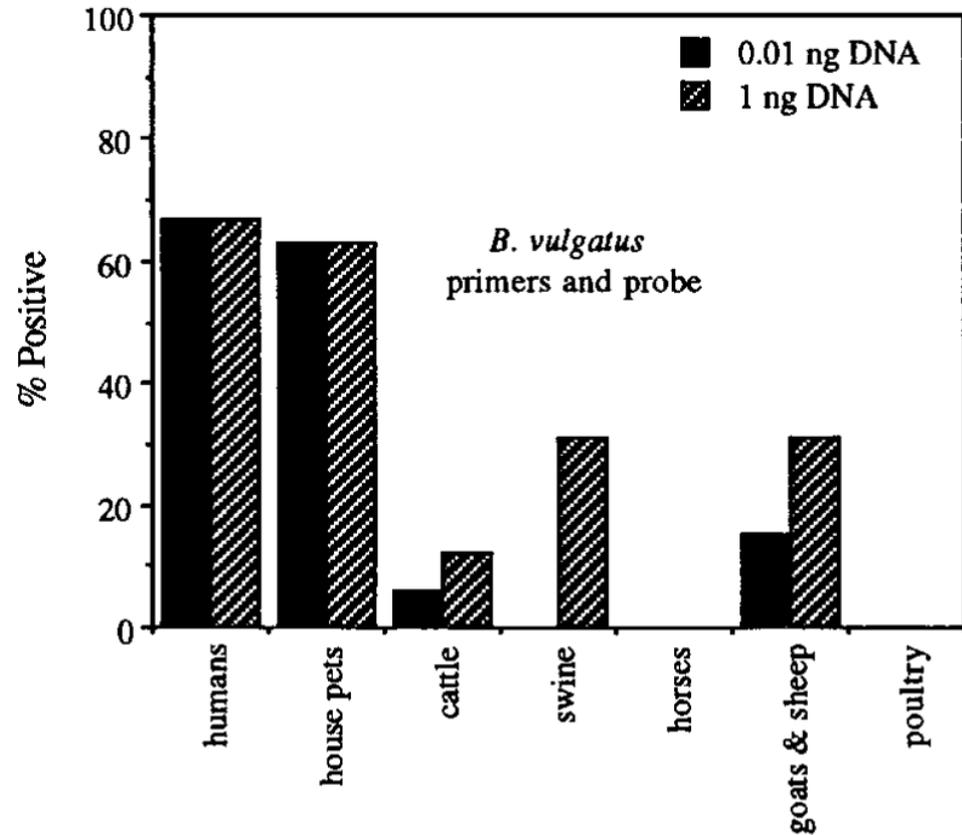


B. fragilis (ATCC® 25285) colonies growing on Brucella Agar. Incubated anaerobically for 24 hours at 35°C.

- Highly abundant in feces and sewage
(~1,000-fold > fecal coliforms)
- Strict anaerobe
- Difficult to cultivate
- Not prevalent in birds
(Fogarty and Voytek, 2005)

Bacteroides Species as a Host-Identifier

- Focus on cultivated species only
- Poor specificity to human waste



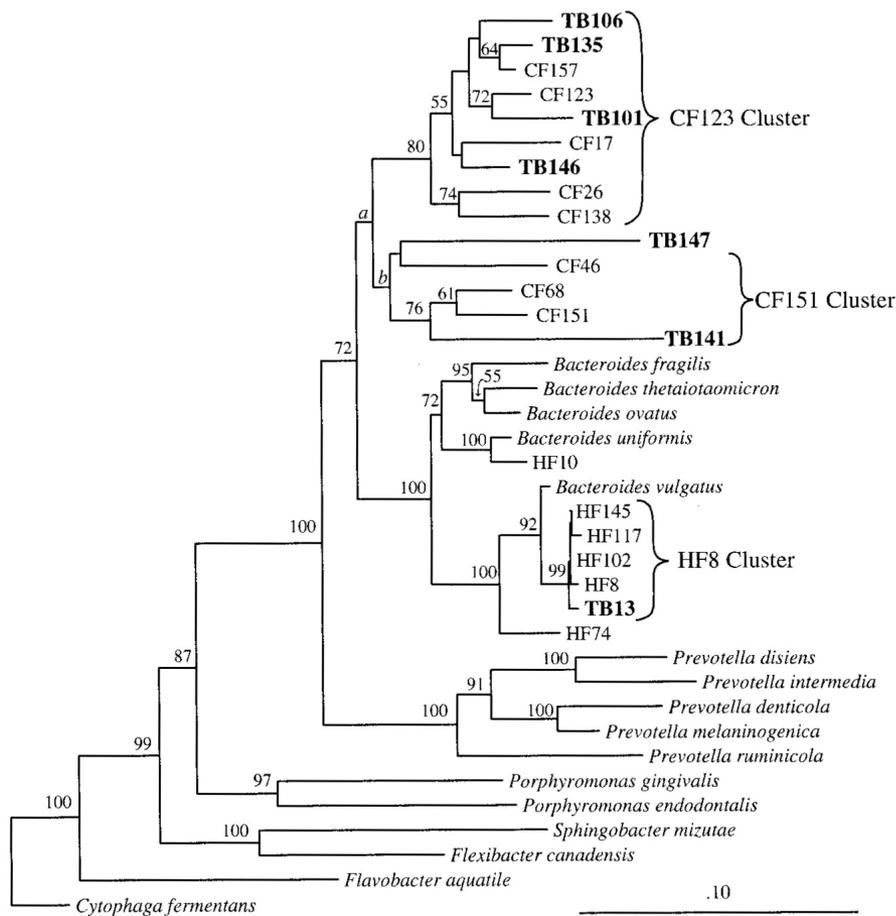
Targeting Uncultivated *Bacteroides* starts Present-Day Microbial Source Tracking Field

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2000, p. 4571-4574
0099-2240/00/\$04.00+0
Copyright © 2000, American Society for Microbiology. All Rights Reserved.

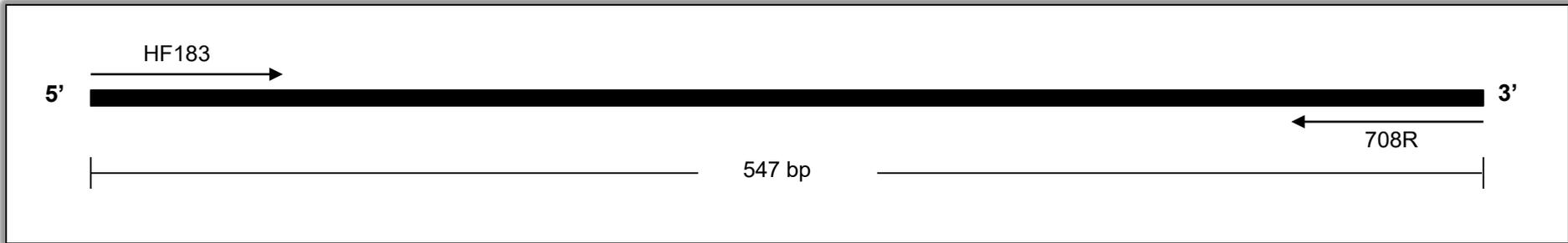
A PCR Assay To Discriminate Human and Ruminant F Basis of Host Differences in *Bacteroides-Prevote* Genes Encoding 16S rRNA

ANNE E. BERNHARD AND KATHARINE G. FIELD*
Department of Microbiology, Oregon State University, Corvallis, Oregon 97330
Received 14 April 2000/Accepted 26 July 2000

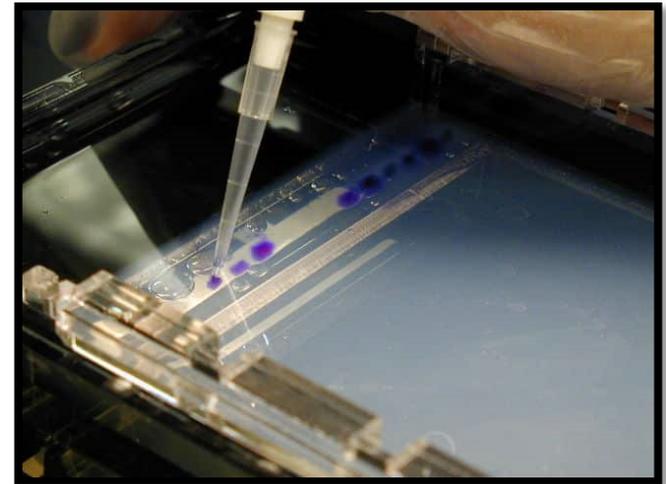
- Novel 16S rRNA sequences from feces and polluted water
- HF8 cluster
- 4% sequence identify to *B. vulgatus*



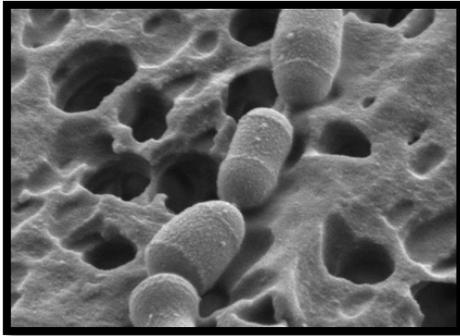
HF183 DNA Target Sequence Anatomy



- End-point PCR platform
- Forward primer HF183
(specificity for human-associated *Bacteroides*)
- Reverse primer 708R
(non-specific to maximize sensitivity)



A *Bacteroides* Strain Bearing the HF183 DNA Target Sequence Isolated in 2006



International Journal of Systematic and Evolutionary Microbiology (2006), 56, 1639–1643

Bacteroides dorei sp. nov., isolated from human faeces

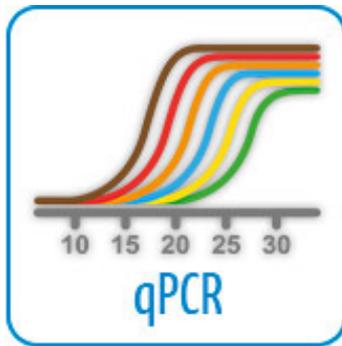
Mohammad Abdul Bakir,¹ Mitsuo Sakamoto,¹ Maki Kitahara,¹
Mitsuharu Matsumoto² and Yoshimi Benno¹

¹Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama 351-0198, Japan

²Laboratory of Dairy Science and Technology, Kyodo Milk Industry Co. Ltd, Hinode, Tokyo 190-0182, Japan

- *B. dorei* isolated from human feces
- 100% sequence identity to HF183 target sequence
- 4% 16S rRNA sequence divergence from *B. vulgatus*

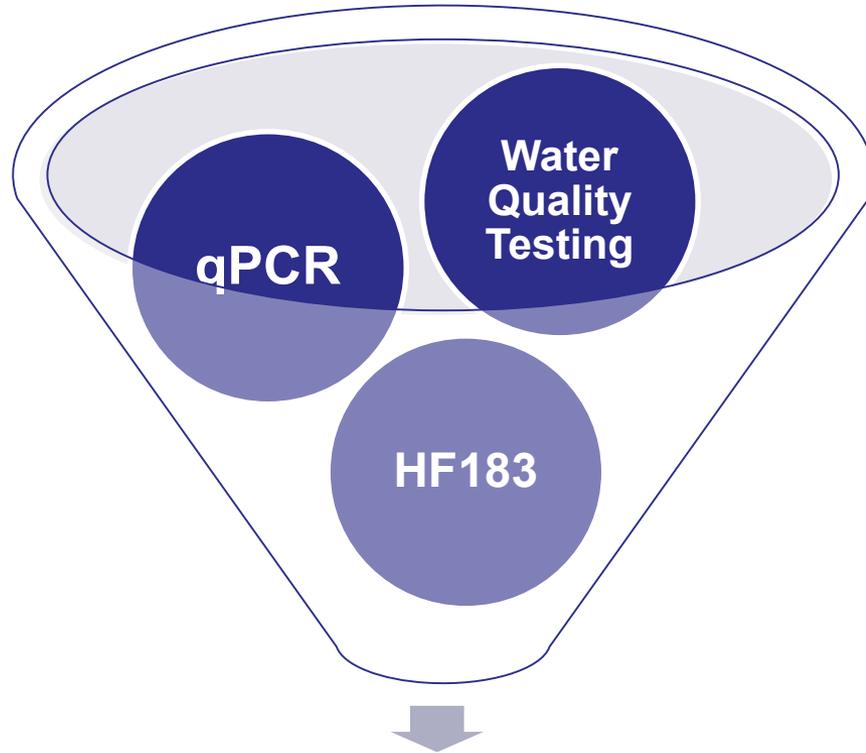
Quantitative PCR Allows Estimation of DNA Target Concentration in Test Sample



- Mainstream scientific technology
- “Gold standard” for many applications
- No cultivation requirement
- Sensitive and specific in complex systems
- Highly reproducible when standardized
- Established quality control guidelines (Bustin et al. 2010)
- Specialized reagents for environmental testing



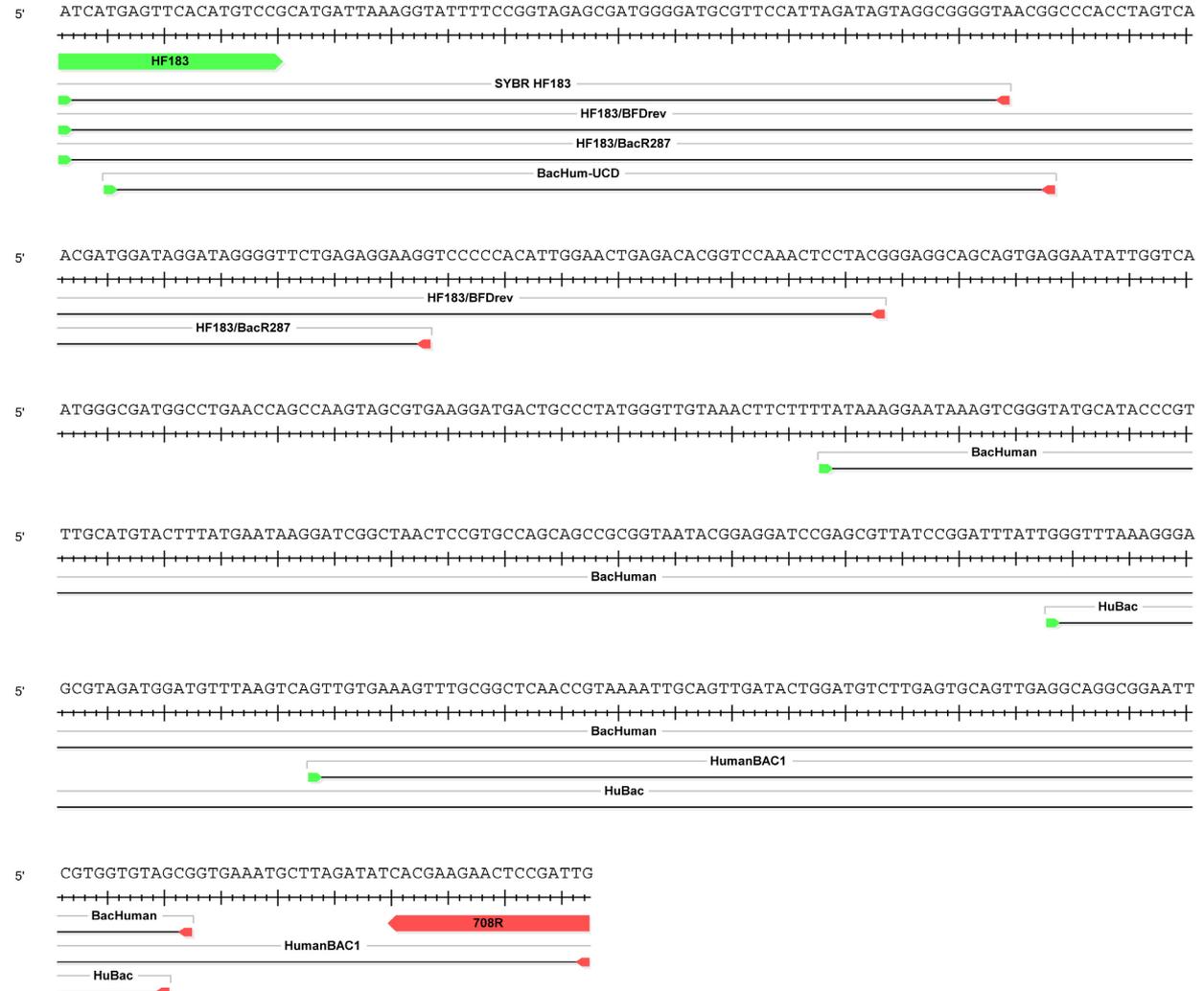
Concept Formulation Review



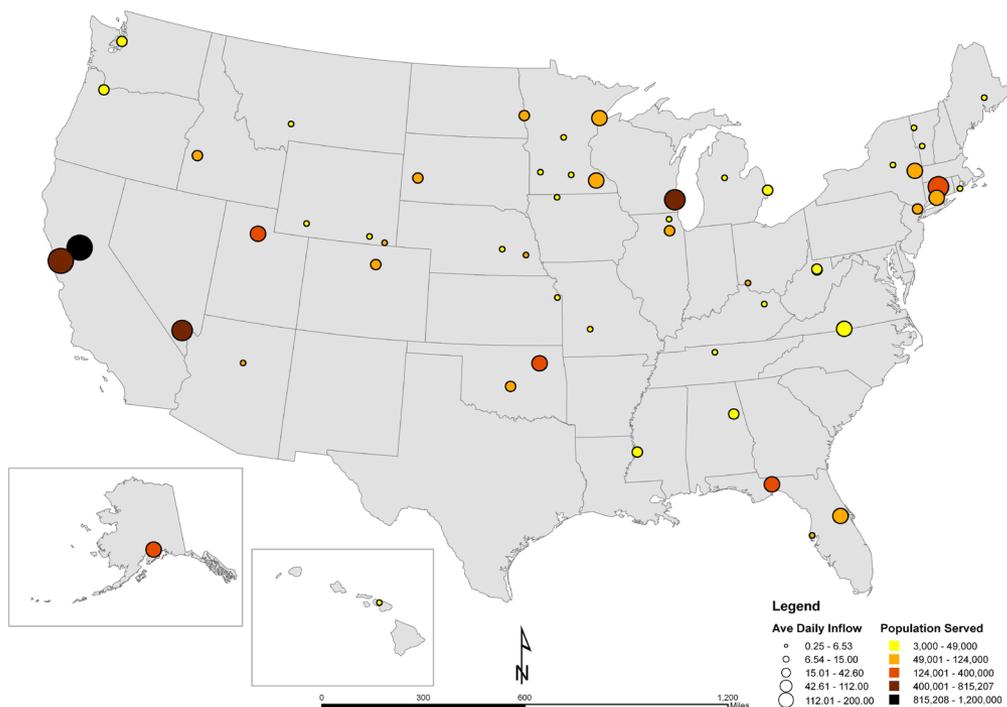
**Quantitative Human-Associated Microbial
Source Tracking Tool for Water Quality
Management**

HF183 Adapted to qPCR Platform

- **HF183 SYBR** (Seurinick et al. 2005)
- **HuBac** (Layton et al. 2006)
- **BacHum-UCD** (Kildare et al. 2007)
- **HumanBAC1** (Okabe et al. 2007)
- **BacHuman** (Lee et al. 2010)
- **HF183/BFDrev** (Haugland et al. 2010)
- **HF183/BacR287** (Green et al. 2014)

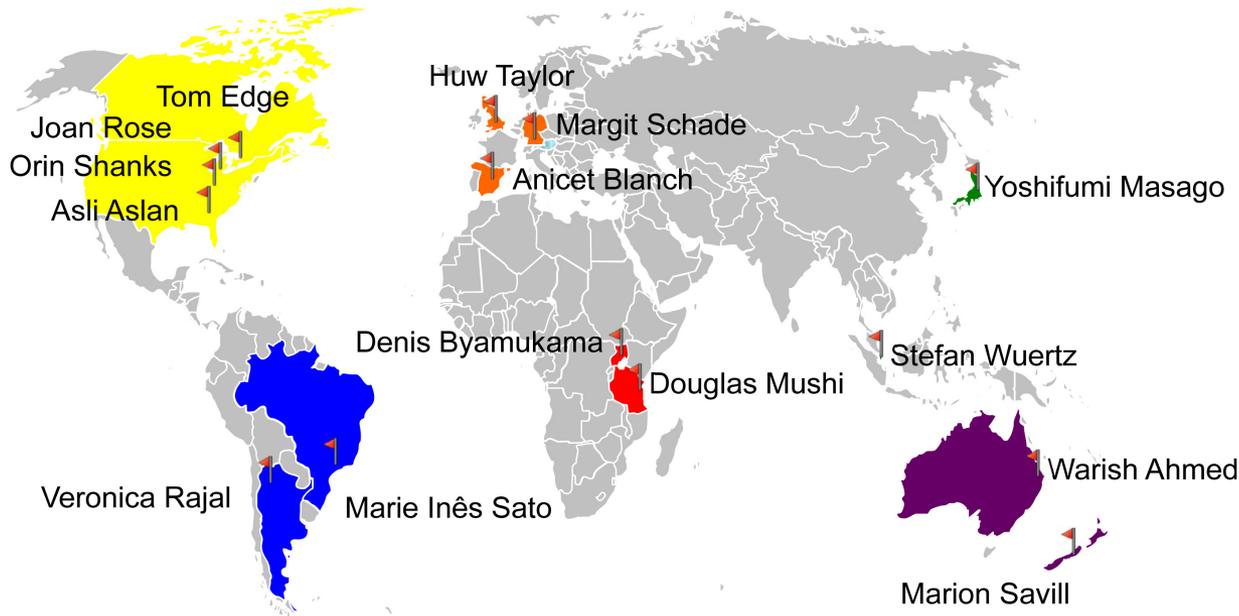


HF183 Broadly Distributed in U.S. Sewage



- Evaluation on national scale
- Sewage reference collection
 - 54 Facilities
 - 39 States
 - 1,150 MGD
 - ~6.4 Million Individuals
- HF183 present at measurable levels in all samples

HF183 Also Found Worldwide



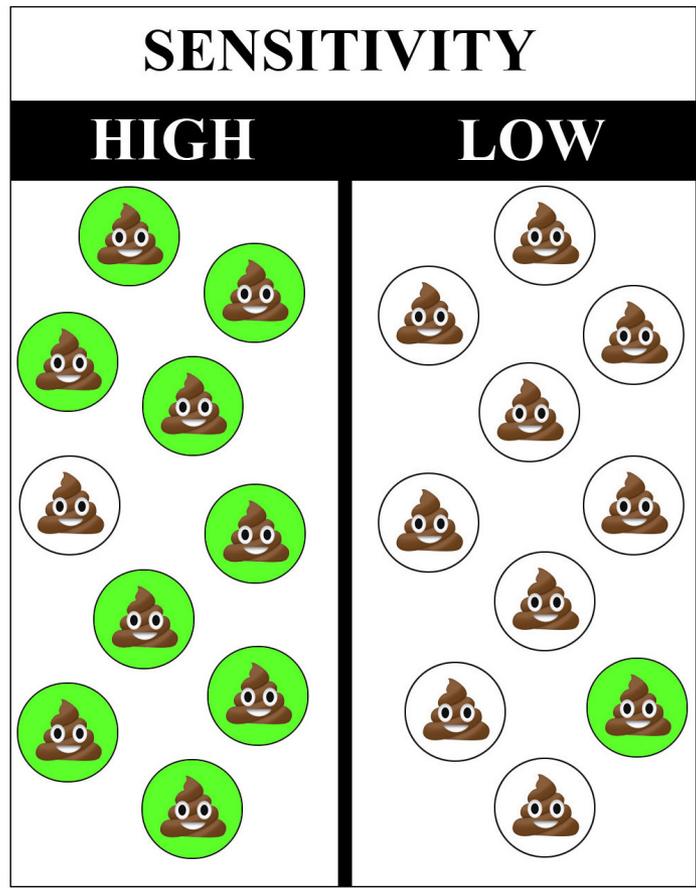
- Argentina
- Australia
- Austria
- Bangladesh
- Belgium
- Brazil
- Canada
- France
- Germany
- India
- Japan
- New Zealand
- Puerto Rico
- Singapore
- Spain
- Tanzania
- Uganda
- United Kingdom
- United States

Mayer et al. (2018). Global distribution of human-associated fecal genetic markers in reference samples from six continents. *Environmental Science & Technology*. IN PRESS

Both Sensitivity and Specificity Are Important for HF183 Performance

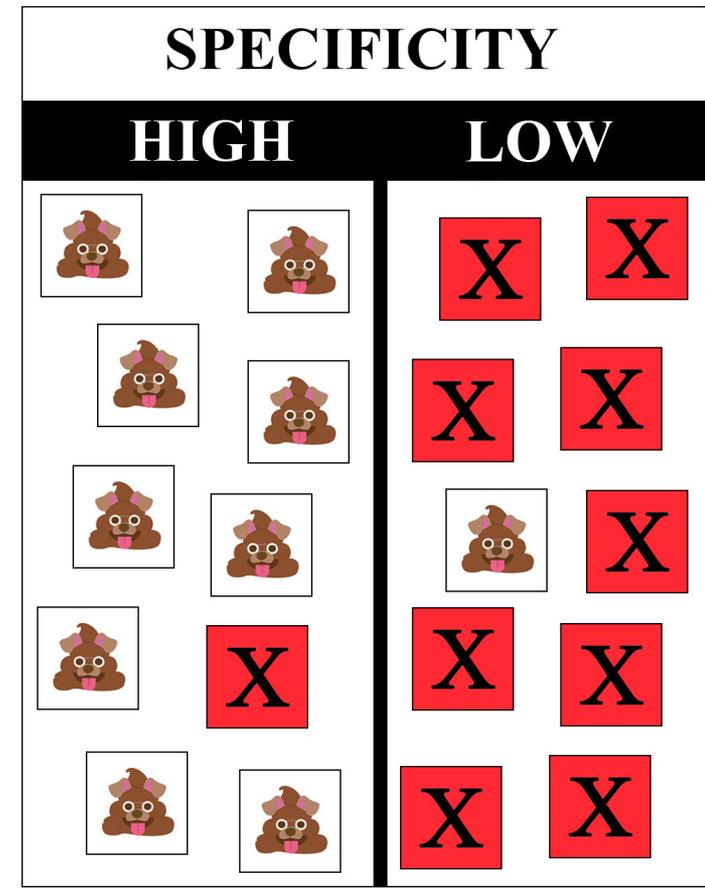
Human
 Human Detection

Non-Human
 False Positive



90%10%

(Human Targets Only)



90%10%

(Non-Human Targets Only)

HF183 Cross-Study Sensitivity and Specificity Performance



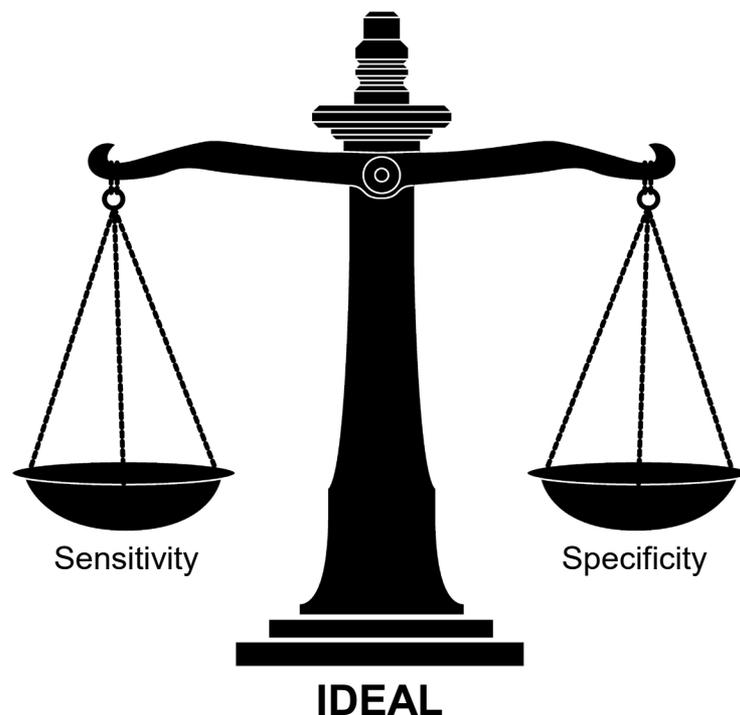
OVERALL SENSITIVITY ESTIMATE

Sewage	Feces
~98% (n=580)	75% (n=142)



REPORTED SPECIFICITY RANGE

60% to 100%
<i>Sporadic detection of cattle, racoon, chicken, dog, deer, rabbit, and gull sources</i>



Lack of Method Standardization Influences Specificity Performance

- Labs often use modified lab protocols

- **Good Practices:**
 - Use exact procedure reported by method developer

- **Bad Habits:**
 - Modify protocol without evidence of equivalent performance

DNA Polymerase

Sample Concentration

Amplification Buffer

Reaction Volume

LIMIT OF DETECTION DEFINITION

Primer/Probe Concentrations

Data Acceptance Criteria

Cycle Number

Reference Library

DNA Extraction Procedure

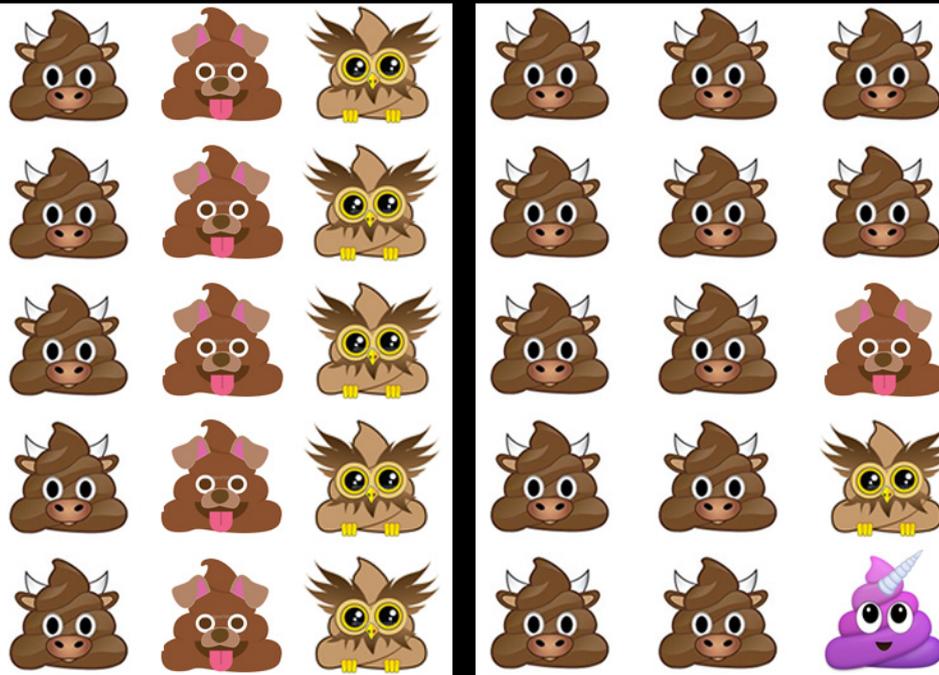
Thermal Cycle Instrument

Specificity Influenced by Reference Library Composition

LIBRARY COMPOSITION

GOOD

BAD



- Each study uses a different reference library

• Good Practices:

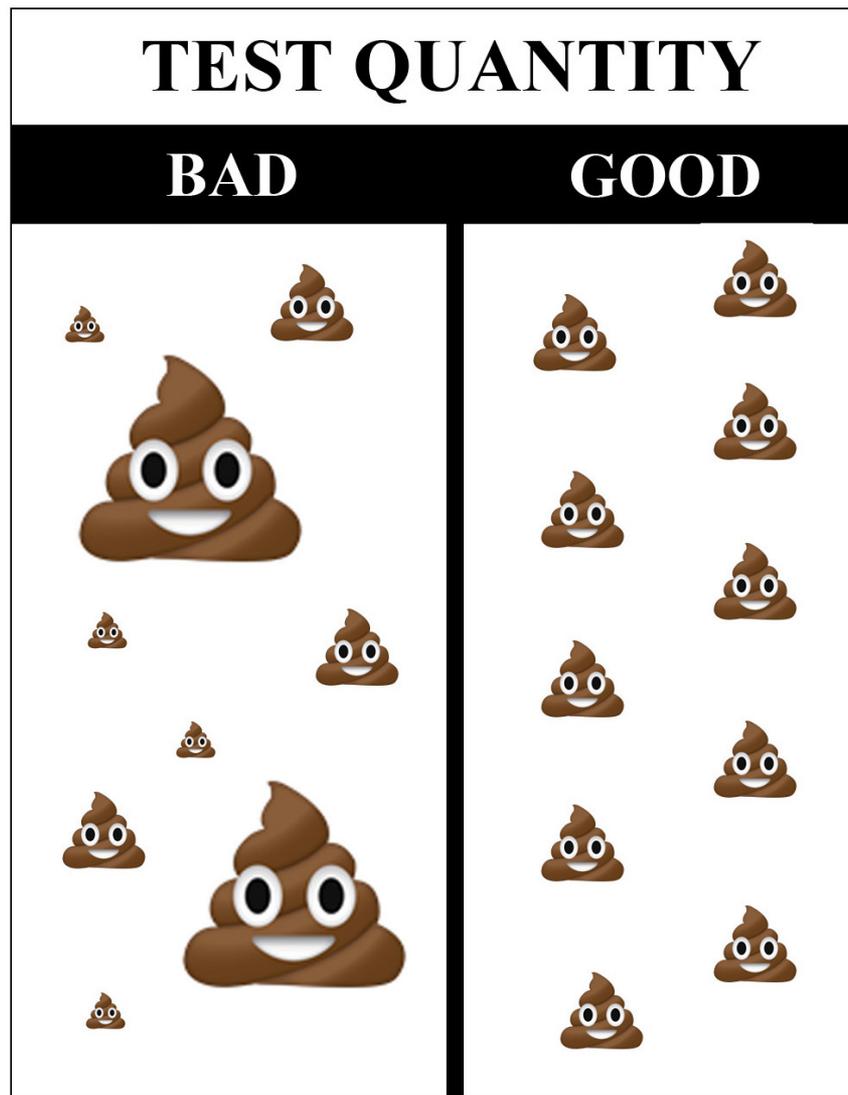
- Equal representation
- Relevant sources only

• Bad Habits:

- Unequal representation
- Low impact source(s)

Specificity Influenced By Sample Test Concentration

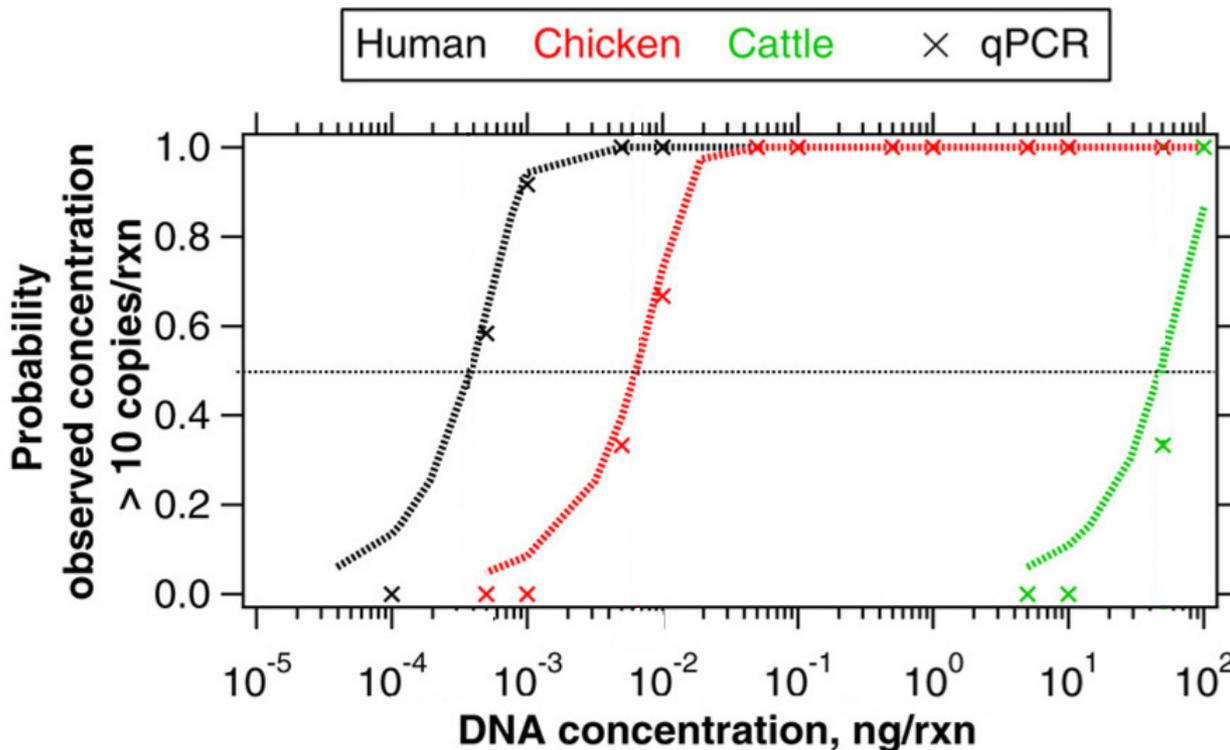
- **Test concentration not standardized between studies**
-
- **Good Practices:**
 - Equal test quantity
 - Report test concentration
 - Use standardized procedure
 - **Bad Habits:**
 - Unequal test quantities
 - Poor methods reporting




Low
Concentration

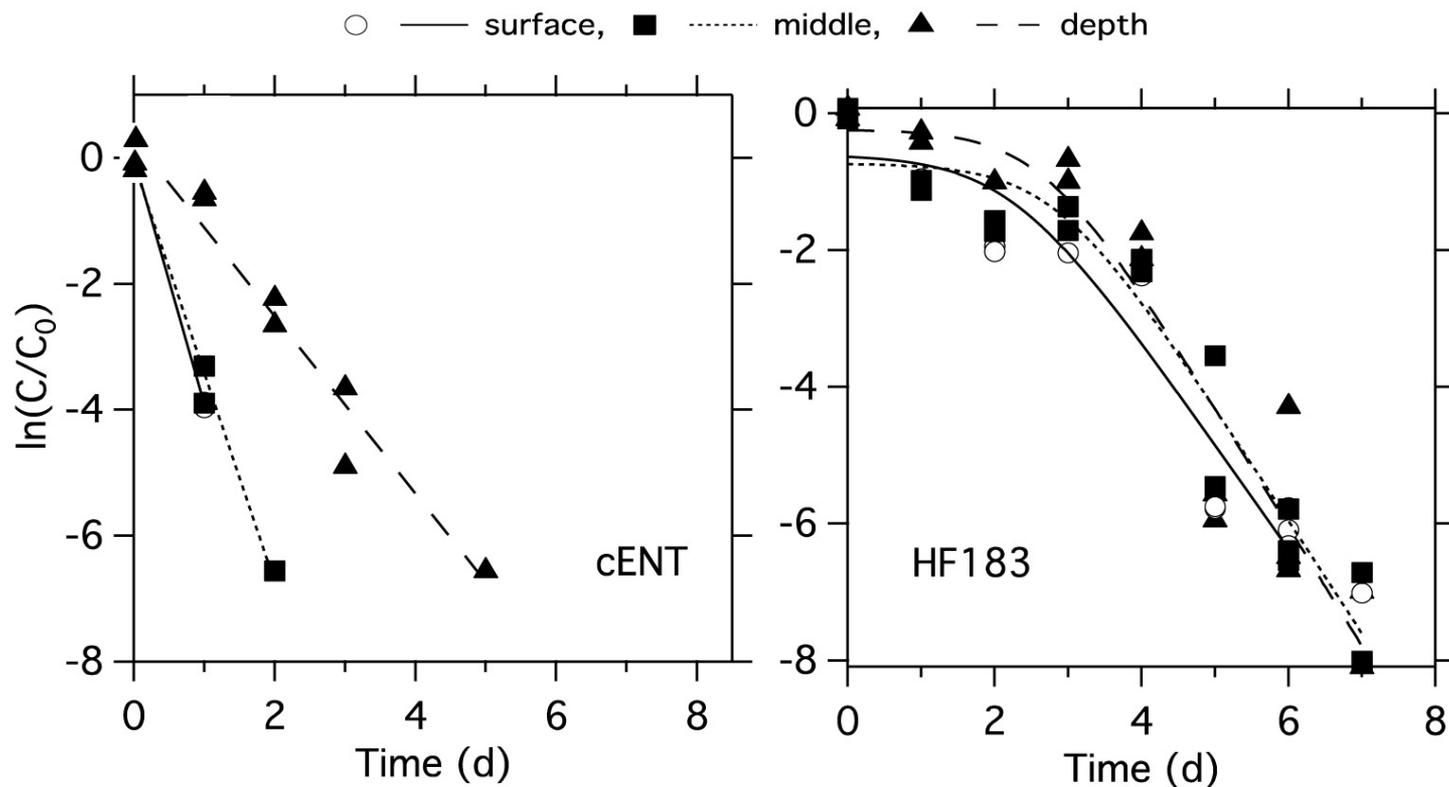

High
Concentration

Test Concentration is Key: A Better Way to Evaluate HF183 Specificity



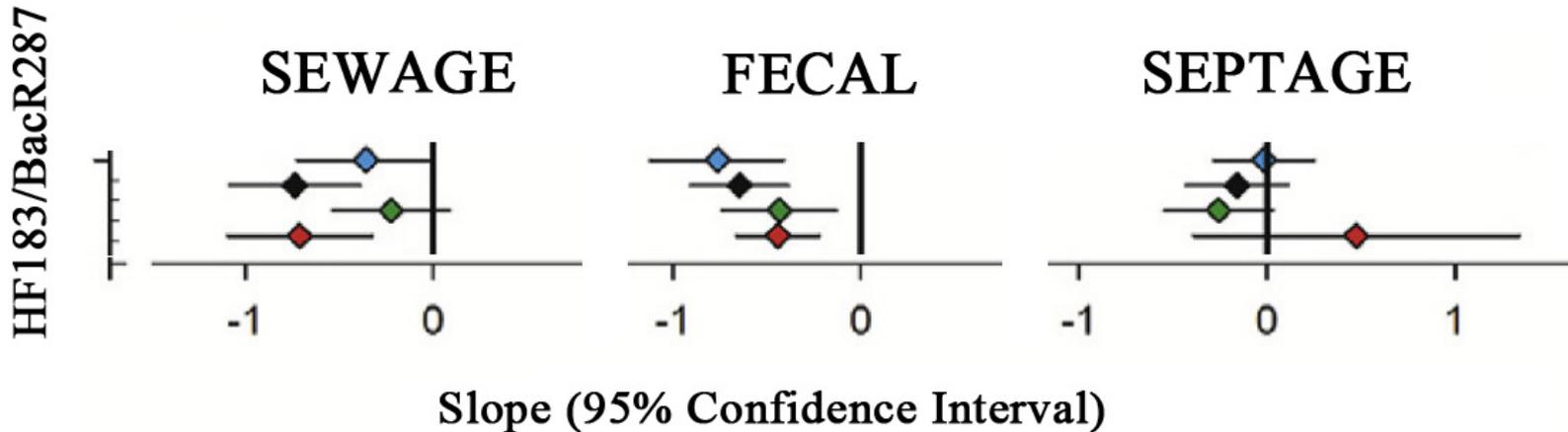
- Probability of qPCR measurement is function of test concentration
- Same dilution pattern across different sources
- HF183 concentration typically lower in non-human sources
- It takes more non-human fecal pollution to generate same result with human source

HF183 Decay ≠ Cultivated Fecal Indicator Bacteria Decay

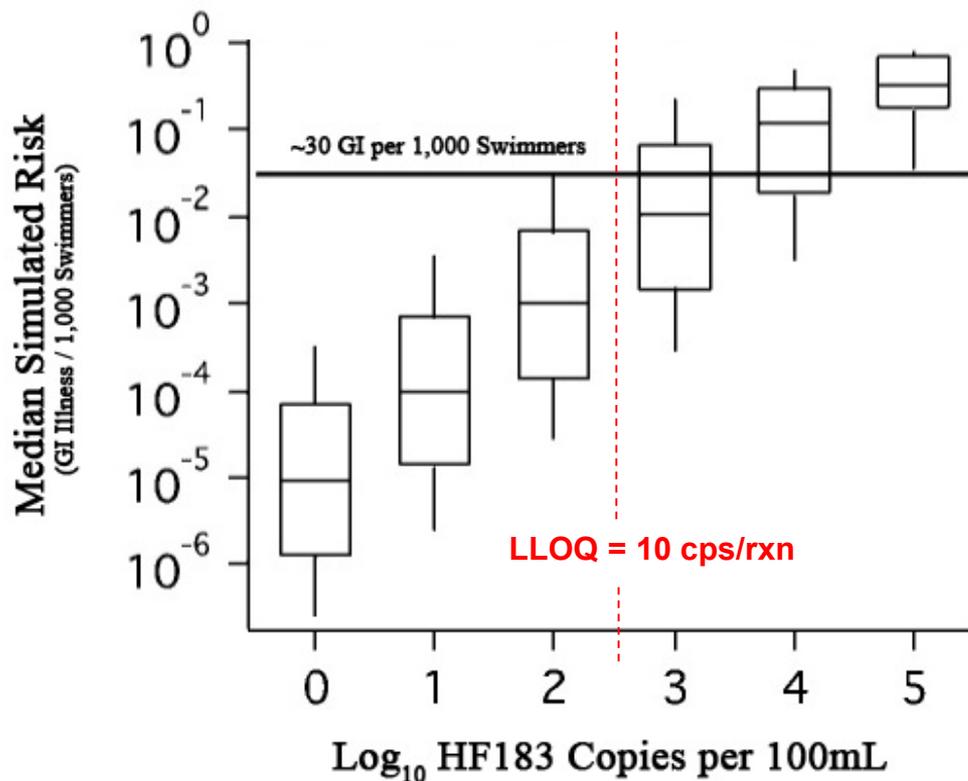


Common Human Fecal Pollution Sources Exhibit Different Decay Trends

- ◆ Treatment A (Sunlight + Indigenous Microbiota)
- ◆ Treatment B (Sunlight + Reduced Indigenous Microbiota)
- ◆ Treatment C (Shaded + Indigenous Microbiota)
- ◆ Treatment D (Shaded + Reduced Indigenous Microbiota)
- Slope = 0



Public Health Risk Based HF183 qPCR Interpretation



Quantitative Microbial Risk Assessment (QMRA) approach

- Raw sewage pollutant scenario
- Positive detections below typical LLOQ may be health risk relevant

HF183 qPCR Used in Diverse Settings

Key Matrices	Example Applications
Urban, Snowmelt, Agricultural Run-Off	Human Non-Point Identification
Recreational Marine/Freshwater	Recreational Site Prioritization for Remediation
Aquaculture Waters	Nutrient Discharge Characterization
Streams, Lakes, Rivers	Waterborne Disease Outbreak Response
Sediments	Urban Stormwater Outfall Management
Beach Sand	Best Management Practice Evaluation
Groundwater	Total Maximum Daily Load Management
Septic System Discharge	Recreational Water Monitoring
Stormwater Outfalls	Shellfish Water Management
Combine Sewer Overflows	Drinking Water Reservoir Protection

Regional Validation of HF183 qPCR

- California 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**
- qPCR methods are highly reproducible across labs only when protocol is **standardized**
- Identification of top human-associated qPCR methods
 - **HF183**
 - **HumM2**



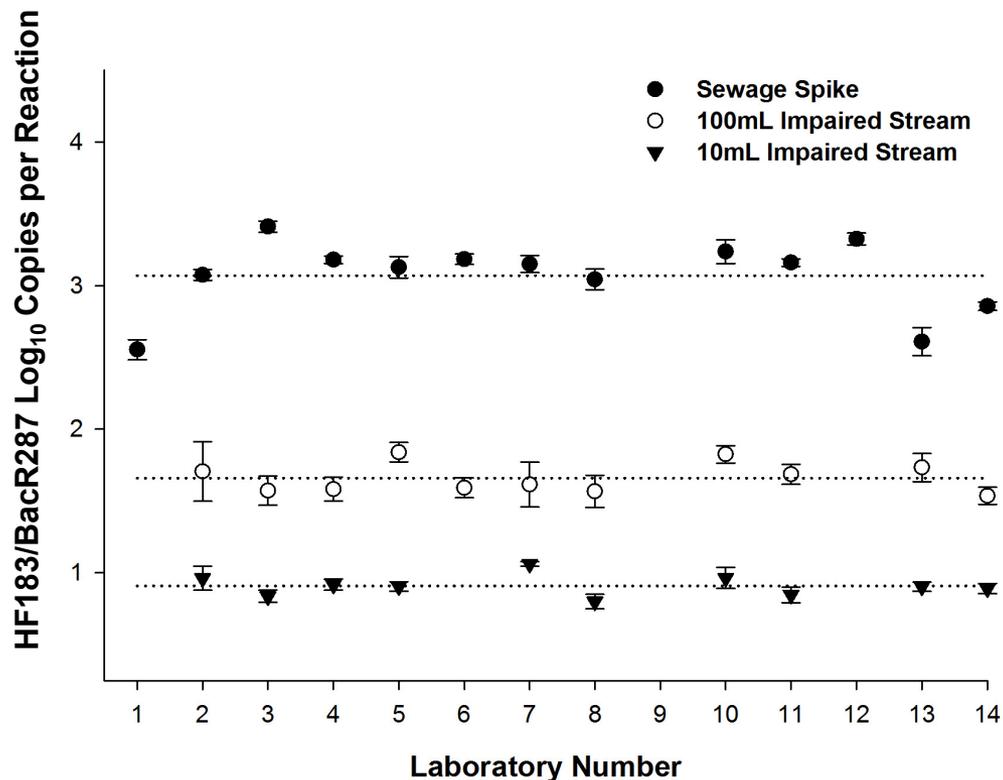
Boehm *et al.* (2013) Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research* 47: 6812-6828.

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.

National Validation of HF183 qPCR



- EPA National Study
 - Office of Water
 - Office of Research & Development
- HF183/BacR287 qPCR
- 14 Lab Participants
- Supplied with:
 - Standard protocols
 - Reference DNA materials
 - Sewage spike material
 - Blinded filter set (n = 18)
 - All reagents and consumables

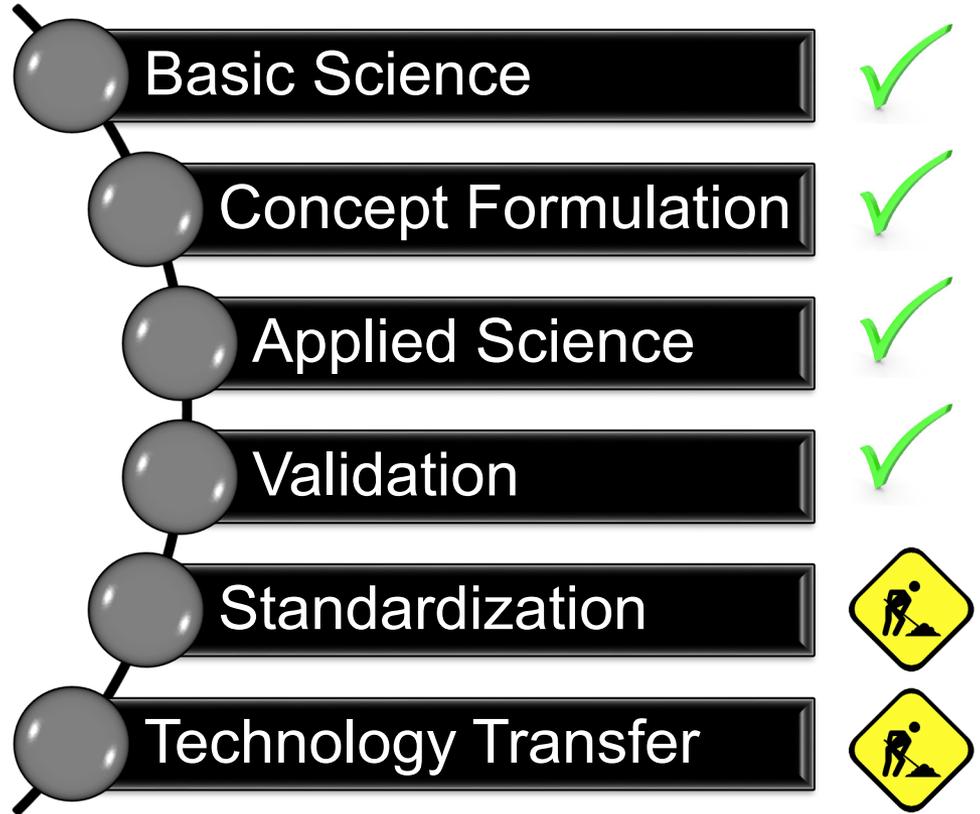
HF183 qPCR Data Acceptance for Management Tool Application

Type	Metric
Calibration Curve Model	Linearity (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)

- Many potential sources of error in qPCR
- Benchmark metrics to ensure acceptable performance
- Required for scientific credibility of findings

HF183 Management Tool Development Progress Review

- Almost 20 years since first described
- Expert consensus 2013
- Many applications
- Increasing demand



What is the Deal with False Positives in Non-Human Sources?

- Are there predictable patterns to HF183 non-human occurrence?
- Recent study reports that gulls ingest human waste (Alm et al. 2018)
- Can some animals with HF183 be potential transport vectors of human pathogens too?
and/or
- Can *Bacteroides* with HF183 colonize a non-human gut?

Verizon 8:11 PM 95%

< POPULAR SCIENCE AA



We're so close with dogs, even our poop looks similar

A new study finds that human and dog microbiomes have more in common than you might expect.

Kat Eschner

Value of Method Standardization and Data Acceptance Criteria



- **Obvious Benefits:**

- Required for multiple lab implementation
- Enhance public acceptance
- Promote data compatibility

- **Less Obvious Benefits:**

- Increases access
- Establishes shortcut for new technology development

One Protocol Does Not Fit All Applications

- Sampling strategies
 - Site selection
 - Sampling frequency
- Data interpretation
 - Ancillary data requirements
 - Additional data analysis procedures
- Resource logistics
 - Local laboratory capacity
 - Leveraging available resources
 - Local partnerships





Some Research Gaps are Application-Specific

Non-Point Source ID

- Standardized methodology
- Standardized DNA reference materials

Spatial-Temporal Trends

- Standardized methodology
- Standardized DNA reference materials

Public Health Risk Indicator

- Standardized methodology
- Standardized DNA reference materials
- Established link to public health risk
- Approved application by regulatory agency

Enforcement

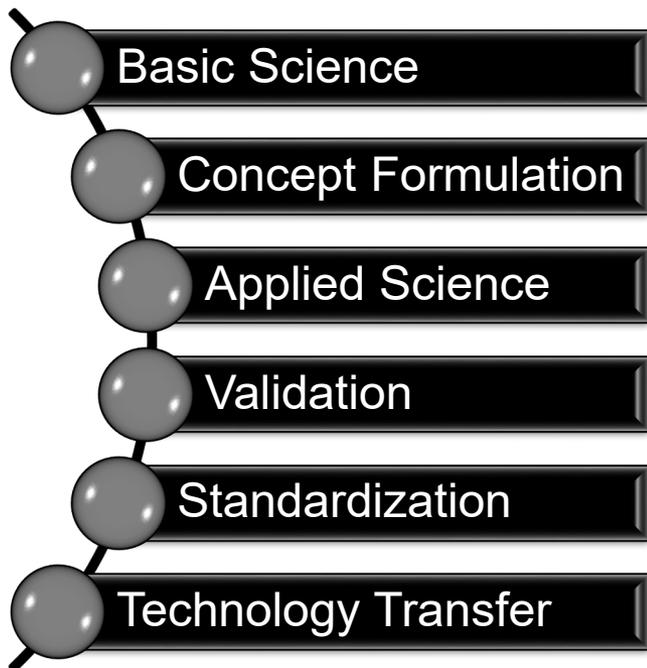
- Standardized methodology
- Standardized DNA reference materials
- Lab accreditation pathway
- Weight of evidence legal definition
- Approved application by regulatory agency

Technology Transfer is a Game Changer

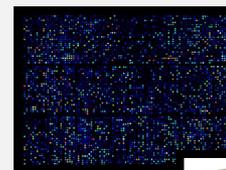


- Develop a Scaling-Up Strategy
- Goals:
 - Widespread implementation
 - Public acceptance
 - Improved feasibility
 - Increased lab capacity
- Technology Transfer Priorities:
 - Centralized standard reference materials
 - Better data visualization and reporting tools
 - Improved communication strategies
 - Training opportunities

How Can We Streamline Future Method Development?



- Implement standardized procedures
- Promote multiple lab studies
- Blueprint for emerging MST technologies



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- David McCarthy, MU

HF183
Management
Tool

Many, many, more ...

QUESTIONS?

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