Microbial Source Tracking with qPCR: Applications and Technology Transfer

*Orin C. Shanks*
Presentation Overview

1. Microbial Source Tracking Background

2. MST qPCR in Action

3. EPA MST qPCR Technology Transfer Activities

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Fecal Pollution is a Nationwide Challenge

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

Top 10 Causes of Impairment in U.S. Rivers and Streams

- Pathogens
- Sediment
- Nutrients
- Oxygen Depletion
- Temperature
- Metals
- Polychlorinated Biphenyls
- Mercury
- Habitat Alterations
- Turbidity

https://ofmpub.epa.gov/waters10/attains_nation_cy.control#causes
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

- 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

- Public health risk can vary by source
- Mitigation strategies can vary by source
- Source information improves management and public safety
A Microbial Source Tracking Solution

**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 Microbial Source Tracking Technologies Available

- Microarray
- Next generation sequencing
- End-point PCR
- Quantitative real-time PCR
- Digital PCR
- Terminal restriction fragment length polymorphism
- Antibiotic resistance analysis
- Chemical detection
- Canine scent detection
Technology 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 qPCR-based technology

- Identification of top methods for pollution sources

Benefits of MST with qPCR

- 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
Many Water Quality Management qPCR MST Applications

- Urban stormwater management
- Impaired site prioritization for remediation
- Evaluation of a best management practices
- Total Maximum Daily Load planning
- Hazardous event response
- Waterborne disease outbreak response
Importance of Field Studies

• One MST qPCR procedure will not work for all applications
  - Sampling strategies
  - Ancillary data requirements
  - Data analysis procedures

• Real-world examples are crucial
  - Application tailored methodology
  - Peer-reviewed

• Implementation Strategy
  - Develop core procedure
  - Conduct field studies
  - Provide tailored methods to public
MST in Action: Identification of Septic Pollution with MST qPCR

Question: Does human fecal pollution originate from leaky sewer lines or failing septic systems in my watershed?

East Fork Little Miami Watershed
- 1,295 km² Southeastern Ohio watershed
- Range of septic/sewer use intensity
- 9 catchment areas
- Small stream sampling
- 24-month sampling period
- 3 human-associated qPCR methods
- Unsafe levels of fecal pollution > 40% of time
  \((E.\ coli\ and\ enterococci\ MPN\ cell\ counts)\)

Quantifying Catchment Land Use with GIS

- Estimate sewer and septic densities
- Normalized by catchment area

Experimental Design to Address Question

- Catchments represent gradient of sewer and septic use

- Negative correlation between septic and sewer densities \( (R^2 = -0.69) \)

- Does human pollution trend with sewage, septic, or neither?

Identifying Human Fecal Pollution Trends

- Human fecal pollution increases with septic density (wet weather events only)
- Trend supported by all 3 human-associated qPCR methods
- Potential Actionable Outcome: septic site inspections

MST in Action: Agriculture and Wildlife Impacts with MST qPCR

Question: Does wildlife and agricultural practices contribute to chronic fecal pollution in my watershed?

Tillamook Basin
- 1,500 km² northern Oregon coast
- Active dairy industry
- 29 catchment areas
- 12-month sampling period
- 8 host-associated qPCR methods
- Chronic fecal pollution
Water Quality Management with *E. coli*
Avian Pollution Spatial and Temporal Trends

Potential bird migration water quality impact
Spatial and Temporal Trends in Other Fecal Sources

• Spatial trends
  - Land use
  - Waste management practices

• Temporal trends
  - Agricultural practices
  - Wildlife activities

• Varies by source
• **E. coli** exceedance (80%)
• Seasonal dog pollution, target local breeding facility
• Possible bird migration impact
• Possible rain event human impact
• Ruminant in spring, likely beef cattle AFO

• Potential actionable outcomes:
  ➢ Site inspection in survey in Spring
  ➢ Target AFO, septic system, and dog facility
Recreational Beach Management with MST qPCR

- Recreational activity annual public health and economic impacts
  - About 90 million illnesses\(^1\)
  - Approx. $2.9 billion medical expense\(^1\)

- Managed with general fecal indicators (\textit{E. coli} or enterococci)
  - Identifies problem
  - No source information

- Control strategies can vary by source

- MST qPCR applications
  - Linking pollution source to general indicator
  - Site prioritization by pollution source

\(^1\) DeFlorio-Barker et al. (2018) Environmental Health 17:3
MST in Action: Recreational Beach Management with MST qPCR

**Question:** Are there any links between my MST qPCR and general indicator measurements?

- University of Illinois at Chicago School of Public Health study
  - Sam Dorevitch (Principal Investigator)
  - Abhilasha Shrestha (PhD Candidate)

- 9 beaches sampled 5 days/week over beach season

- *E. coli* and enterococci general indicator testing

- MST qPCR testing for human, bird, and dog sources
Linking General Indicator and MST Findings

Enterococci qPCR Weighted-Averages

- **Group #1:** ≥ 1,000 CCE (US EPA recommended BAV)
- **Group #2:** < 100 CCE

1. Shrestha et al. manuscript in preparation

- Group samples based on local recreation criteria
- Calculate weighted-average for each group
- Compare differences between groups:
  - Bird 8.4x higher
  - Dog 4.2x higher
  - Human similar
- Potential actionable outcomes:
  - Minimize bird activity
  - Restrict dog access
MST in Action: Recreational Beach Management with MST qPCR

Question: How do I prioritize sites based on human fecal pollution levels?

- Partners:
  - City of Racine Health Department
  - Northeast Ohio Regional Sewer District
  - Scientific Methods, Inc.

- 6 sampling sites

- Potential pollution sources (human, bird and dog)

- Sampled 5 days/week over beach season

- 16 water quality and beach area parameters
• > 80% of U.S. population live in communities with MS4 discharges
• 7,550 regulated communities
• MS4 permittees required to develop, implement, and mitigate stormwater management programs
• MS4 discharges can contain fecal waste
• Control strategies can vary by source
Growing Interest in MST qPCR and Urban Stormwater Management

- Charles River and Boston Harbor  
  (Boston Water and Sewer Commission)

- City of Santa Barbara  
  (State Clean Beach Initiative)

- Hampton Roads Sanitation District  
  (Virginia Beach, VA)

- Oklahoma Stormwater Quality Program  
  (City of Tulsa Streets and Stormwater Dept)

- Colorado *E. coli* Toolbox: A Practical Guide for Colorado MS4s (Urban Drainage & Flood Control District City and County of Denver)
MST in Action: Urban Stormwater Management with MST qPCR

**Question:** What are the sources of fecal pollution in my MS4 outfalls?

- **Partners:**
  - Department of Energy & Environment
  - ORISE
  - EPA Region 3 Laboratory

- 7 first order catchments
- 32 MS4 outfalls
- Routine and event sampling
- Potential pollution sources (human, ruminant, bird and dog)
MST qPCR: Implementation Status

- Many examples in scientific literature
- No nationally standardized methods or application guidance yet
- Some qPCR MST methods closer to “prime time” than others
  - Human > Ruminant, cattle > swine > dog > avian
- Recommend confirming performance with local reference samples
- Ideal to consult expert for assay selection, experiment design, and result interpretation
- Need for improved data visualization and communication tools
EPA qPCR MST Technology Transfer Activities

- National validation of two human-associated qPCR methods
- Towards standardized EPA Methods
- Development of implementation tools
- EPA outreach activities
EPA Multiple Laboratory Validation - Overview

- Formal study conducted by EPA
  - Office of Water
  - Office of Research & Development

- Two qPCR Methods

- 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
Draft EPA Methods 1696 and 1697: Content Overview

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

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

- Microsoft Excel
- Standardized input
- Summary report
Self-Administered 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
Reference DNA Material Development

- National implementation requires a 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
EPA Outreach Activities

- Building a support network
- Communication
- Training opportunities
- Cooperative partnerships
  - States, tribes, and other local labs
  - Association of Public Health Laboratories MOU
  - Federal agencies
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QUESTIONS?

Orin C. Shanks, Ph.D.
Senior Research Geneticist
Email: shanks.orin@epa.gov
Phone: (513) 569-7314

U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268