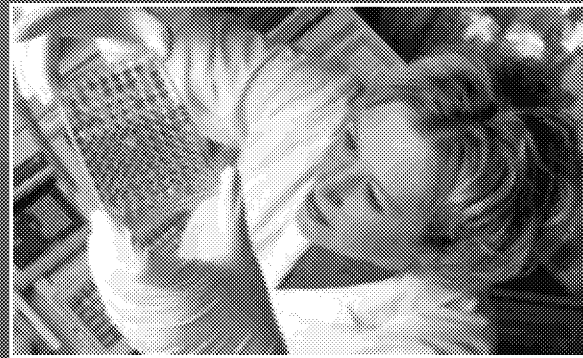


# Innovations in Microbial Water Quality Monitoring: *A Recreational Waters Case Study*



**Kevin Oshima**

*US EPA ORD's National Exposure Research Laboratory*

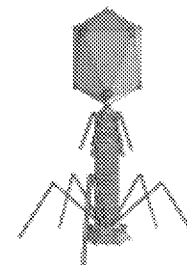
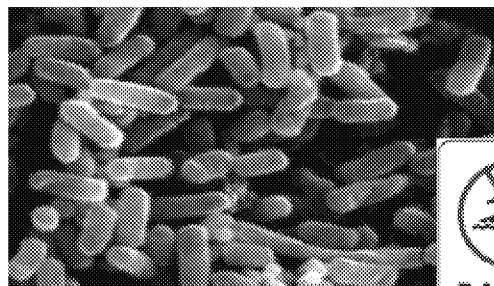
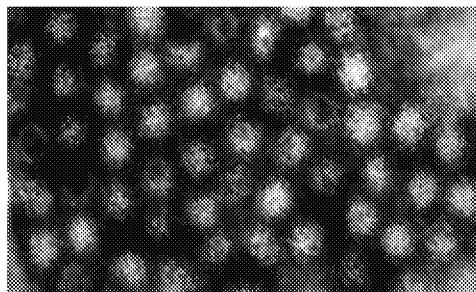
**Region 5 and Neighboring States' Visit to US EPA Cincinnati  
February 2, 2018**

Office of Research and Development

# Problem

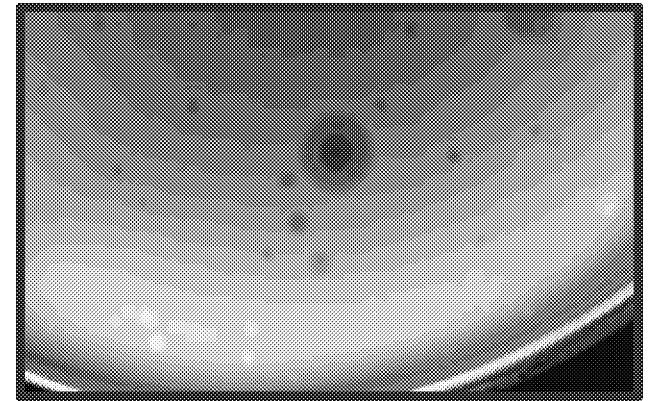
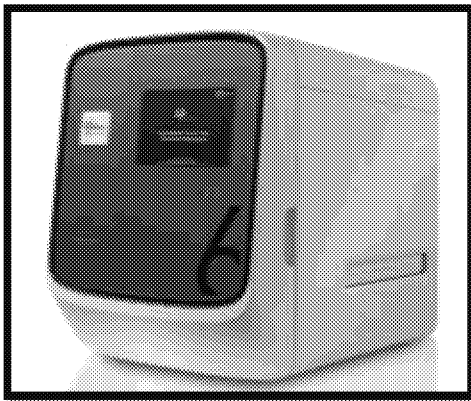
Swimming in recreational waters poses gastrointestinal illness risks to swimmers from fecal pollution → need for improved monitoring tools in the toolbox (tens of millions of surface water associated swimming illnesses in the US a year resulting in billions of dollars in economic burden)

- Direct detection of pathogens is a challenge
- Pollution regulated with general fecal indicators
  - Culture-based methods require overnight incubation
  - Same day, rapid methods improve public safety
- Health risks originating from fecal sources are not equal
  - Human and cattle fecal waste are of highest risk to humans
- Bacterial indicators may not be suitable for viral pathogens which are believed to be the major cause of illness



# Approach

- Recreational Water Quality Criteria (US EPA's Office of Water, 2012 update)
- ORD in Cincinnati focused on research in 3 areas:
  - Rapid Quantitative Polymerase Chain Reaction (qPCR) methods for enterococci and *E. coli* fecal indicator bacteria
  - qPCR microbial source tracking for human fecal contamination
  - Coliphage (a type of bacteriophage, viruses that infect bacteria) as an alternative virus-based indicator of fecal contamination



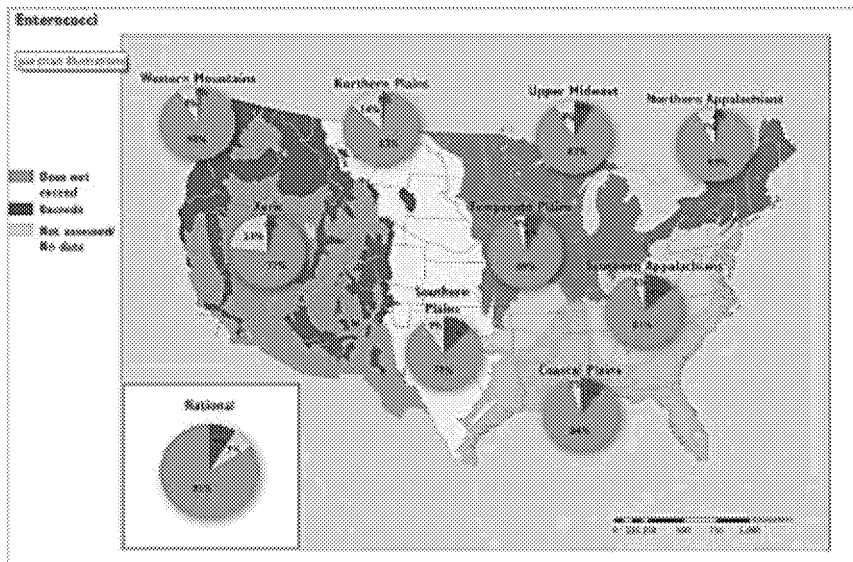
# Improve Methods for Detection of Bacterial General Fecal Indicators

- Development of rapid molecular methods for enterococci and *E. coli*
- Assess method robustness across the US
- Develop criteria for *E. coli* qPCR
- Provide implementation assistance for stakeholders
- Develop microbiological water quality forecasting tool (Virtual Beach)

**Virtual Beach** is a software package designed by US EPA for developing site-specific statistical models for the prediction of pathogen indicator levels at recreational beaches.

Available:

<https://www.epa.gov/exposure-assessment-models/virtual-beach-vb>



**National Rivers and Streams Assessment**

Reprinted from US EPA Office of Water and ORD *National*

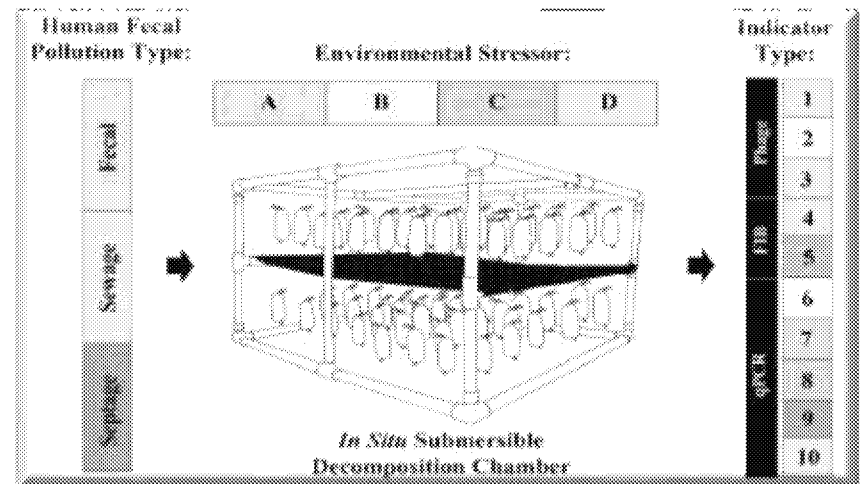
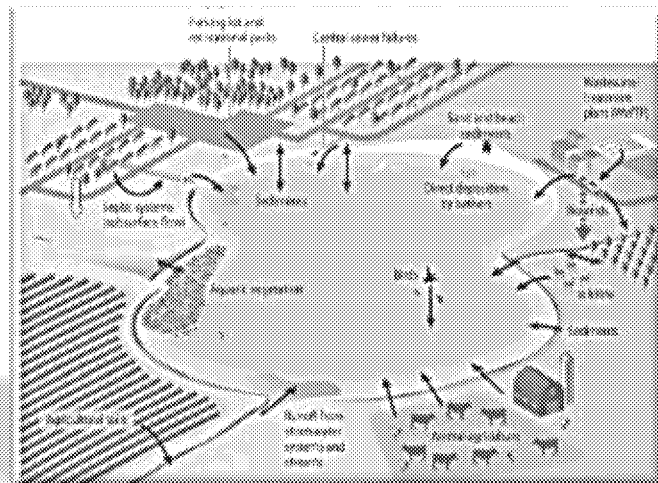
*Rivers and Streams Assessment 2008-2009: A Collaborative Survey (EPA/841/R-16/007)*. Washington, DC. March 2016.

# Microbial Source Tracking Innovative Methods

- Development of human-associated microbial source tracking qPCR methods
  - Method selection by expert consensus
  - Standardization of procedures
  - Build laboratory capacity (training and standardized reagents)
  - Science to support implementation
  
- Case Studies
  - Great Lakes Beaches study
  - Chicago Beaches study
    - Assessment at 9 beaches
    - Collaboration with University of Illinois at Chicago

# Coliphage

- Develop method to detect coliphage in ambient waters
  - Development/validation, performance across the US
- Determine occurrence in wastewater treatment and surface waters
  - Great Lakes Beaches study
- Fate and transport in wastewater treatment and environment
  - Comparisons to other indicators and viral pathogens



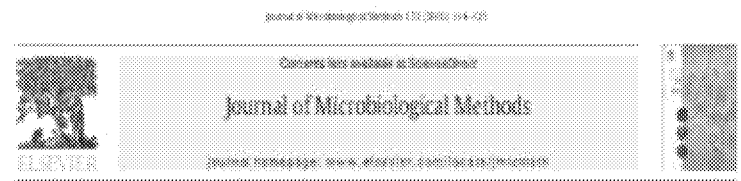
# Results: Improvements in enterococci and *E. coli* qPCR

- US EPA method 1609.1 (enterococci) and draft US EPA method C. (*E. coli*)
  - Validation studies for enterococci (completed) and *E. coli* (pending)
- Building stakeholder laboratory capacity
  - Training, collaboration with Michigan DEQ
- Development of DNA reference standards and standardization of methods
- Development of site specific *E. coli* qPCR criteria for beach notification
  - Collaboration with Michigan DEQ



## Interlaboratory Comparison of Real-Time PCR Protocols for Quantification of General Fecal Indicator Bacteria

Orin C. Shanks<sup>1\*</sup>, Manoj Sivaganesan<sup>1</sup>, Lindsay Feed<sup>1</sup>, Catherine A. Kelly<sup>1</sup>, A. Denise Blackwood<sup>1</sup>, Monica R. Greene<sup>1</sup>, Rachel T. Noble<sup>1</sup>, Rebecca N. Bushon<sup>2</sup>, Erin A. Stecker<sup>3</sup>, Julie Kintzman<sup>4</sup>, Tamara Anan'eva<sup>1</sup>, Christopher Sirigallano<sup>1</sup>, David Winters<sup>1</sup>, John Griffiths<sup>5</sup>, Yiping Cao<sup>5</sup>, Steve Weisberg<sup>6</sup>, Valerie J. Harwood<sup>7</sup>, Christopher Staley<sup>8</sup>, Kevin H. Odama<sup>9</sup>, Manjiv Varma<sup>9</sup>, and Richard A. Hargland<sup>9</sup>



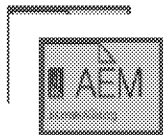
## Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters

Richard A. Hargland<sup>1\*</sup>, Sharen Steffing<sup>2</sup>, Manjiv Varma<sup>3</sup>, Kevin H. Odama<sup>4</sup>, Manoj Sivaganesan<sup>5</sup>, Yiping Cao<sup>6</sup>, Meredith Naird<sup>7</sup>, John Griffiths<sup>8</sup>, Stephen B. Weisberg<sup>9</sup>, Rachel T. Noble<sup>10</sup>, A. Denise Blackwood<sup>11</sup>, Julie Kintzman<sup>12</sup>, Tamara Anan'eva<sup>13</sup>, Rebecca N. Bushon<sup>14</sup>, Erin A. Stecker<sup>15</sup>, Valerie J. Harwood<sup>16</sup>, Katrina V. Gordon<sup>17</sup>, Christopher Sirigallano<sup>18</sup>

# Results: Development and Validation of Human-Associated Microbial Source Tracking Methods

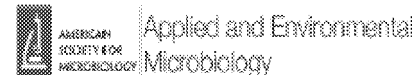
## Human-associated qPCR methods for recreational water (for 2 types of bacteria, HF183 and HumM2)

- Standardized procedure
- Automated data analysis tool
- Data acceptance metrics
- Strong human-association
- US EPA Region 5 lab training
- Reference DNA material
- Quantitative methods
- Expert consensus
- Field demonstrations
- Implementation of tool by stakeholders
- Michigan DEQ lab network training



### Improved HF183 Quantitative Real-Time PCR Assay for Characterization of Human Fecal Pollution in Ambient Surface Water Samples

Hyatt C. Green,<sup>a</sup> Richard A. Haugland,<sup>b</sup> Manju Varma,<sup>c</sup> Hara T. Miller,<sup>c</sup> Mark A. Borcard,<sup>d</sup> Katherine G. Field,<sup>e</sup> William A. Waters,<sup>f</sup> R. Knight,<sup>g,h</sup> Manoj Sivaganesan,<sup>g</sup> Catherine A. Kelly,<sup>g</sup> Orrin C. Stanks<sup>g</sup>



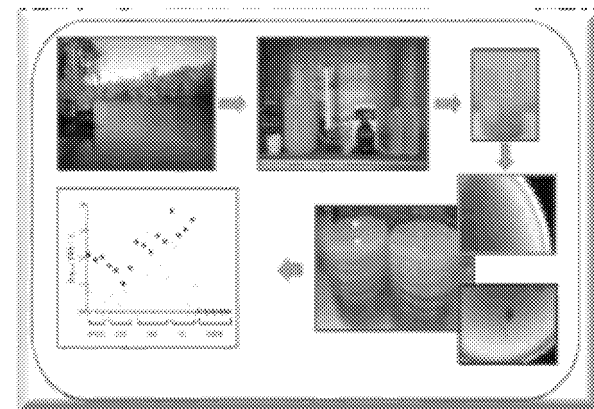
### Data Acceptance Criteria for Standardized Human-Associated Fecal Source Identification Quantitative Real-Time PCR Methods

Orrin C. Stanks,<sup>a</sup> Catherine A. Kelly,<sup>a</sup> Robin Oshiro,<sup>b</sup> Richard A. Haugland,<sup>b</sup> Tania Wladj,<sup>c</sup> Lauren Brooks,<sup>d</sup> Katherine G. Field,<sup>e</sup> Manoj Sivaganesan<sup>g</sup>

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# Results: Coliphage

- Coliphage method development for fresh and marine surface waters and wastewater
  - Ultrafiltration to concentrate 1L samples followed by cultivation
  - Method validation (pending)
  - National assessment of method performance (pending)
- Coliphage fate and transport
  - Published results from OH and FL locations
  - Great Lakes Beaches study (pending)





# Building Stakeholder Laboratory Capacity

US EPA Workshops and Collaboration with Michigan DEQ

- ***E. coli* and enterococci qPCR**
  - Training of labs on EPA Method 1609.1 for Enterococci Draft Method C for *E. coli* qPCR
  - Multi-lab validation study on the *E. coli* method
  - Study to assess the development of criteria for *E. coli* qPCR at Michigan Beaches and impact of applying enterococcus qPCR EPA Method 1609.1
  
- **Human associated source tracking markers**
  - Planning has been initiated for lab training on human-associated qPCR methods (HumM2 and HF183)

## Laboratory Training Course on EPA Methods 1609 and 1611

Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR)

May 12– 16, 2014

EPA Andrew W. Breidenbach Environmental Research Center

26 W. Martin Luther King Drive

Cincinnati, OH 45268

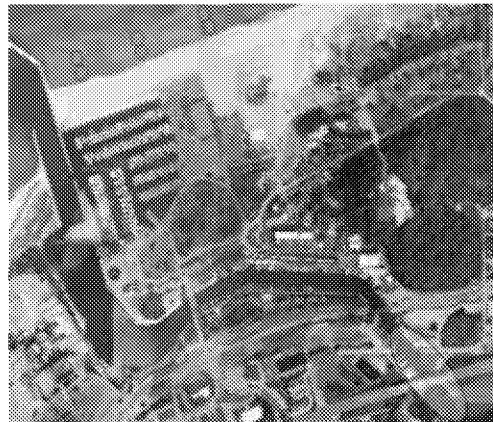
# Indicator Occurrence: Great Lakes Field Studies (Ongoing)



Edgewater Beach, Cleveland, OH



Grant Park Beach,  
S. Milwaukee, WI



Washington Park Beach, Michigan City, IN

- Three Great Lakes beach areas
- Water data collection:
  - Enterococci and *E. coli* qPCR
  - Enterococci and *E. coli* culture methods
  - Microbial source tracking qPCR
  - Coliphage
- 16 water quality and recreational area parameters
- Method comparisons
- Multiple publications FY 2018-19

# Impact: Contributions to Recreational Water Criteria and Applications Toolbox



- Overall Impact
  - Improve microbiological water quality at beaches
  - Better inform when beaches should open or close
- qPCR for enterococci and *E. coli*
  - A tool that improves beach manager's ability to close and open beaches in a more timely manner. Enterococcus qPCR has a stronger relationship to GI illness than overnight culture methods
- Microbial Source Tracking
  - Provides a tool for prioritization of beaches for remediation by identifying beaches that have the greatest human derived contamination
- Coliphage
  - A tool that targets viral indicators and has a potential to improve wastewater treatment and beach monitoring based on close relationship with viral pathogens



# Contact

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