



## **Microbial Source Tracking Process and Applications**

US EPA Region 4 Beach Program  
Managers Meeting  
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# Overview

- Background
- About Microbial Source Tracking
- Testing Considerations
- Project Case Study
- Summary

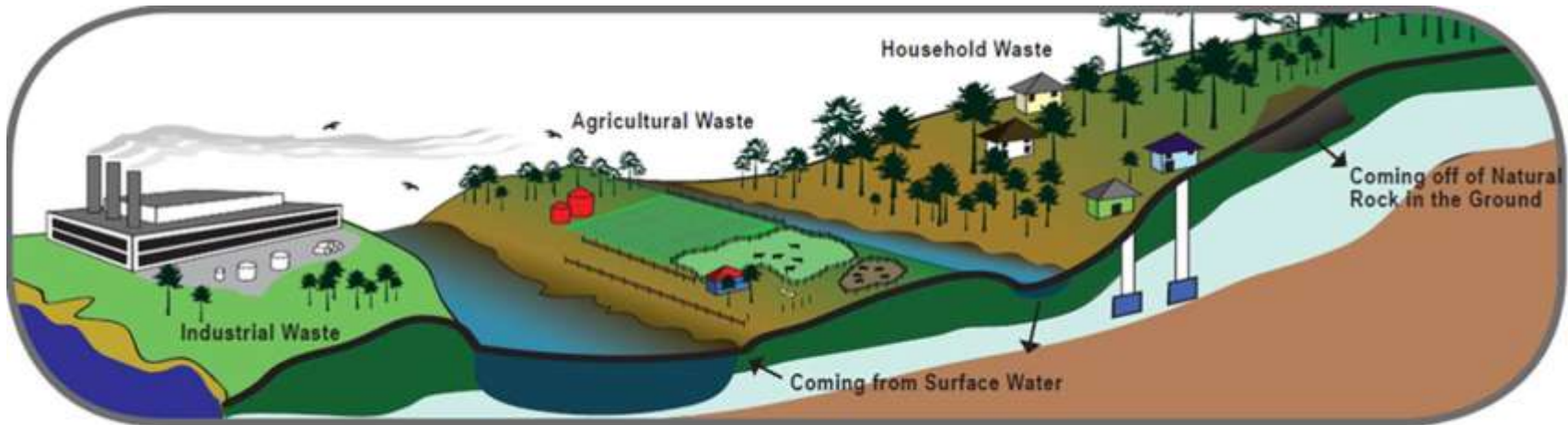
# Water Safety

- Maintaining safe recreational and drinking water to protect public health
- Water Types
  - Beaches, Lakes, Rivers, Reservoirs, and Groundwater
- EPA's Clean Water Act
  - Develop Total Maximum Daily Load (TMDL) for pathogens
- Fecal pollution major contributor of pathogens
  - Causes adverse risks to swimming, fishing, and boating

# Fecal Indicators

- Used to determine the presence of pathogenic organisms
- Typical Fecal Indicators
  - E. Coli
  - Enterococcus
  - Fecal Coliform
  - Bacteroidales (EPA Method B)
- Useful for the detection of **general** fecal contamination
- Provide no insight into the potential sources of fecal contamination

# Pathways for Contamination



(NC Department of Health and Human Services, 2011)

- Industrial Plants
- Farm waste
- Urban runoff
- Sewage
- Septic Leaks
- Wildlife

# Pathways for Contamination



**A Sanitary Survey may be sufficient**

# Microbial Source Tracking

- Host gut conditions and natural selection create unique genetics between host microbial communities.
  - Cow and Chicken microbes communities are not the same
- Genetic differences are identified and used as the basis for fecal host discrimination
- Host specific genes are detected using qPCR
  - Exponentially copies the sequence of interest.
  - Monitors and calculates the starting quantity



# Microbial Source Tracking





# What hosts can MST detect?

## Microbial Source Tracking by Host

Human



Cattle



Swine



Bird



Seagull



Goose



Chicken



Dog



Deer



Horse



- Human
- Cattle
- Swine
- Bird
- Seagull
- Goose
- Chicken
- Dog
- Deer
- Horse
- Additional hosts in development
  - Ruminant and Duck

# Presence/Absence MST Testing

Source Molecular Corporation  
Leader in Genetic Microbial Source Tracking

4985 SW 74th Court, Miami, FL 33155 USA  
Tel: (1) (786) 220-0379, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

## Human Fecal Toolbox ID™

Detection of the Fecal Human Gene Biomarker for Human Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: ABC Company  
Date Received: October 3, 2011  
Date Reported: October 11, 2011

SM #	Client #	Analysis Requested	DNA Analytical Results
SM 16294	01012011A	Human Bacteroidetes ID	Negative
SM 16295	01012011B	Human Bacteroidetes ID	Negative
SM 16296	01012011C	Human Bacteroidetes ID	Positive
SM 16297	01012011D	Human Enterococcus ID	Positive
SM 16298	01012011E	Human Enterococcus ID	Positive
SM 16302	01012011F	Human Enterococcus ID	Positive
SM 16300	01012011A	Human Urine Virus ID	Negative
SM 16301	01012011B	Human Urine Virus ID	Negative
SM 16302	01012011C	Human Urine Virus ID	Positive
SM 16303	01012011D	Human Urine Virus ID	Positive
SM 16304	01012011E	Human Urine Virus ID	Positive
SM 16305	01012011F	Human Urine Virus ID	Positive

- Determines the presence or absence of host specific fecal indicators
- Two tests targeting the same host of interest (e.g. Human) allow confirmation of results, particularly negative ones.
- Valuable for screening multiple hosts prior to quantification
- Less expensive and simpler than quantification

# “Quantification” MST Testing

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**Human Fecal Pollution Quantification ID™**  
Detection and Quantification of the Fecal Human Gene Biomarker for Human Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: ABC Company  
Date Received: October 3, 2011  
Date Reported: October 11, 2011

SM #	Client #	Analysis Requested	General Marker Quantified*	Human Specific Marker Quantified*	DNA Analytical Results
SM 16297	01012011D	Human Bacteroidetes ID	4.57E+03	9.66E+01	Positive
SM 16298	01012011E	Human Bacteroidetes ID	5.44E+03	3.44E+02	Positive
SM 16300	01012011A	Human Urine Virus ID			Negative
SM 16301	01012011B	Human Urine Virus ID			Negative
SM 16302	01012011F	Human Bacteroidetes ID	5.55E+03	1.29E+02	Positive
SM 16302	01012011C	Human Urine Virus ID	5.97E+02	1.58E+02	Positive

\*Numbers reported as copy numbers per 100 mL of water

- Reports the genetic copies from host specific fecal indicators
- These values are used to interpret the host's relative contribution to fecal contamination
- Results reported as:
  - Negative
  - Trace
  - Minor Contributor
  - Major Contributor
- A general fecal indicator can also be quantified to determine the general contamination
- Gives a better understanding of which hosts to target during remediation

SM #	Client #	Approximate Contribution of Human Fecal Pollution in Water Sample	Comment
SM 16297	01012011D	Major Contributor	High levels of human biomarker detected
SM 16298	01012011E	Major Contributor	High levels of human biomarker detected
SM 16300	01012011A	Negative	Negative for human biomarker
SM 16301	01012011B	Negative	Negative for human biomarker
SM 16302	01012011F	Major Contributor	High levels of human biomarker detected
SM 16302	01012011C	Major Contributor	High levels of human biomarker detected

# Project Case Study

- **Problem**

- State of Delaware was observing high levels of Enterococcus at an estuarine inland recreational beach

- **Goal**

- Determine if Human(HF183 & HPyVs), Dog or Seagull fecal sources contributed to the high Enterococcus levels

# Project Case Study

## ○ Plan

- Use MST quantification tests for Human, Dog, Bird and Seagull to determine which host was contributing to fecal contamination
- 49 Samples taken through the summer months and sent to Source Molecular for analysis
  - Equal number of samples analyzed by USF
  - Samples taken in knee deep water 3 times a week at 3 locations

# Project Case Study

## ○ Results

### ○ Dog

- 4% of the samples were positive
  - Quantification values showed few dog specific genetic copies

### ○ Human Bacteroidetes (HF183)

- 19% of the samples were positive
  - Quantification values showed few human specific genetic copies

### ○ Seagull

- 98% of the samples were positive in high levels
  - Quantification values showed abundant seagull specific genetic copies

# Project Case Study

## ○ Conclusions

- Seagull was likely a major contributor the fecal pollution detected at the beach
- Human a possible contributor but occurring sporadically
- Dog not a likely fecal pollution contributor



# Project Case Study

## ○ Concerns and Considerations

- Are markers available for the potential fecal sources in the water system
- Inter-laboratory variability can make result comparisons a challenge
- Cross reactivity for markers must be taken into account
- Is the sampling plan and MST analysis protocol appropriate to answer the question?
- What is the scale and budget for the project
- Microorganisms used for MST markers may not have a direct correlation to traditional fecal indicators.

# About Source Molecular

- Established in 2002
- Fill void between research and implementation
- Resource for implementing a Microbial Source Tracking project
- Active in the research and development of new approaches
- Strict focus on Microbial Source Tracking
- Pending ISO 17025 accreditation

# Summary

- Water quality and regulatory compliance main concerns
- Fecal indicators useful for general detection but not source identification
- Microbial Source Tracking a tool to supplement routine testing and sanitary surveys
- Detection of human, cattle, swine, bird, gull, goose, chicken, dog, deer, and horse possible
- A well designed study is critical to have to most value from Microbial Source Tracking



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**Case Study: Microbial Source Tracking at Tower Road Bayside Beach, Rehoboth Bay, Sussex County, Delaware 2011 (Dr. Humphries, DE DNREC; Dr. Harwood, USF; and Mr. Larenas, Source Molecular)**