



**Microbial Source Tracking:  
In Search of Human, Gull, and  
Dog**

12th Annual Great Lakes Beach  
Association Conference  
October 16-18, 2012

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## Microbial Source Tracking In Search of Human, Gull, and Dog

Fecal pollution problem

Microbial source  
tracking plan

Submitting and testing  
samples

Project results and  
outcomes

Conclusion

# 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 pollution
- ▶ Provide no insight into the potential sources of fecal pollution

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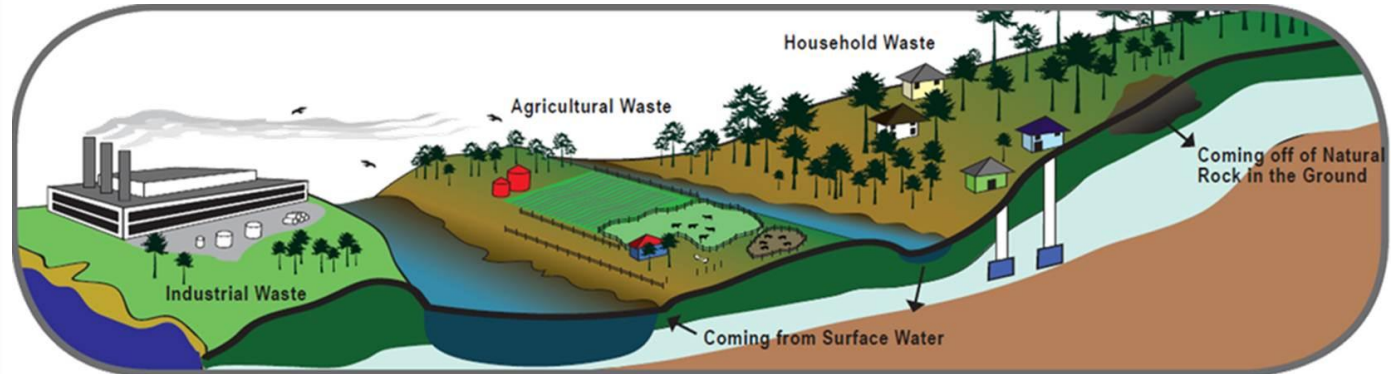
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# Pathways for Contamination



(NC Department of Health and Human Services, 2011)

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

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# Project Case Study

## Problem

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



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## Goal

- ▶ Determine if Human, Dog or Seagull fecal sources contributed to the high Enterococcus levels

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# Funding

## Funding source for Delaware project:

- ▶ EPA Region 3 BEACH Act grant
- ▶ Delaware Environmental Laboratory

## Michigan funding:

### Nonpoint Source Program Grants

- ▶ **DESCRIPTION:** Michigan's Department of Environmental Quality administers a Nonpoint Source (NPS) Program that assists state, federal, and local partners to restore water bodies impaired by NPS pollution and protect high quality waters from impairments caused by NPS pollution.

### Michigan Sea Grant

- ▶ **DESCRIPTION:** Michigan Sea Grant, a cooperative program of the University of Michigan and Michigan State University, is part of the National Sea Grant College Program. It aims to promote better understanding, conservation and use of Michigan's coastal resources. Michigan Sea Grant funds integrated research projects designed to foster science-based decisions about the use and conservation of Great Lakes resources.

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# Validation of local fecal samples

## Validate the sensitivity of two biomarkers

### Human Polyomavirus- USF

- ▶ Raw untreated wastewater from 3 wastewater treatment facilities in Sussex County, DE
- ▶ **Results**
- ▶ Sewage tested positive for HPyVs

### Gull Biomarker - USF/EPA

- ▶ 37 fecal samples from herring and/or laughing gulls tested individually
- ▶ **Results**
- ▶ 84% of the samples tested positive for the gull associated marker

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## Plan

- ▶ Use 1 Seagull, 1 Dog, and 2 Human tests to determine which host(s) 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



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## Plan

- ▶ DNREC would test for general fecal indicator
  - ▶ Enterococcus concentrations - Enterolert®

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# Submitting Samples

- ▶ Samples were filtered at the Delaware DNREC laboratory and submitted frozen on dry ice
- ▶ Samples typically submitted as 500mL water samples overnight
- ▶ Sterile bottles and packing material provided in most cases
- ▶ Serves as convenience and to maintain QA/QC

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# Microbial Source Tracking

Host gut conditions and natural selection create unique genetics between host microbial communities.

- ▶ Cow and Chicken microbial 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

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# What hosts can MST detect?



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# Presence/Absence MST Results

- ▶ **Turnaround time: 5-10 working days**
- ▶ Determines +/- of host specific fecal indicators
- ▶ Multiple tests targeting the same host (e.g. Human) allow confirmation of results
- ▶ Valuable for screening multiple hosts prior to quantification
- ▶ Less expensive than quantification

  
**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

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# “Quantification” MST Results

- ▶ **Turnaround time: 5-10 working days**
- ▶ 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



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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	5.65E+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

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

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## Results

- ▶ **Dog**
  - ▶ 4% of the samples were positive
    - ▶ Quantification values showed few dog specific genetic copies
- ▶ **Human Bacteroidetes (HF183)**
  - ▶ 8% of the samples were positive
    - ▶ Quantification values showed few human specific genetic copies
- ▶ **Seagull**
  - ▶ 98% of the samples were positive
    - ▶ Quantification values showed abundant seagull specific genetic copies

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## Project conclusions and outcomes

- ▶ Seagull was likely a major contributor the fecal pollution detected at the beach
- ▶ Human was a possible contributor but occurring sporadically
- ▶ Dog was not a likely fecal pollution contributor
- ▶ The purpose of the MST study was to confirm the visual observations that seagulls contributed to the elevated levels of Enterococcus (Delaware's water quality indicator) at this beach. Based on MST results, there will be no change to management actions at the site.



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# Project Considerations

- ▶ Are markers available for the potential fecal sources in the water system?
- ▶ Are the markers properly validated?
  - ▶ Cross reactivity and sensitivity for those markers
- ▶ What is the scale and budget for the project?
- ▶ Microbes used for MST markers may not have a direct correlation to traditional fecal indicators

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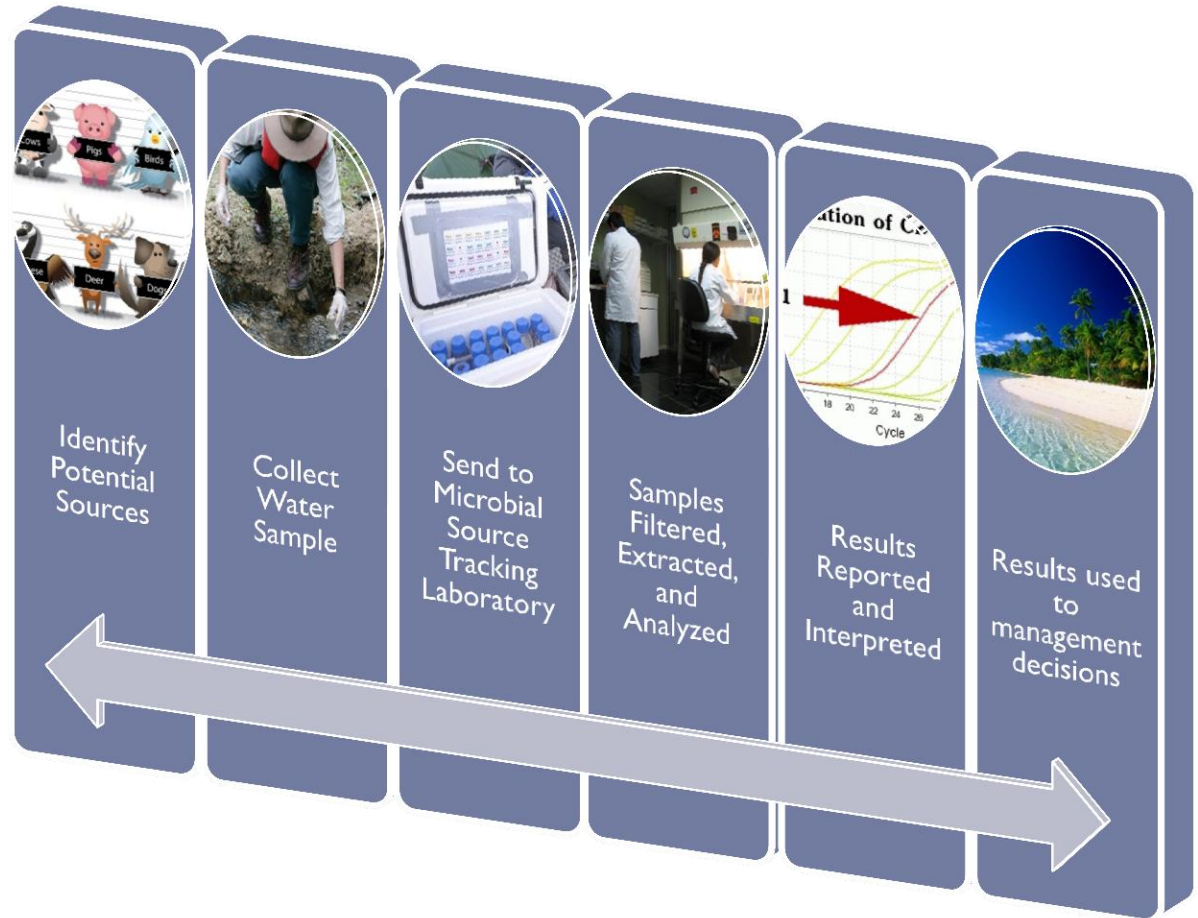
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# About Source Molecular

- ▶ Established in 2002 to fill the void between research and implementation
- ▶ Active in the research and development of new approaches
- ▶ Strict focus on Microbial Source Tracking
- ▶ Detection of human, cattle, swine, bird, gull, goose, chicken, dog, deer, ruminant and horse possible
- ▶ Pending ISO 17025 accreditation



# Source Molecular

Leader in 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)