Evidence-based Guidelines for Microbial Source Tracking Projects

September 5th, 2018
22nd Annual Conference of INAFSM
Evansville, IN

Providing Genetic & Analytic Solutions for Water

Accredited* Water DNA Lab

*World's only ISO 17025 Accredited MST Lab

Project & Site Analytics

Digital PCR

Pathogens (BSL2)

Nutrient Source Tracking

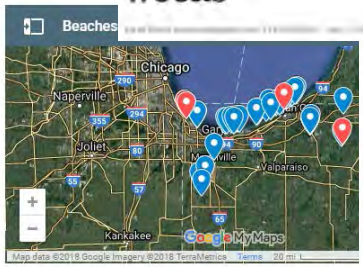
Host Fecal Score

QUIT

Question?
Uninterested at this time
Information, please.
Time to Talk!

Increasing pressure to “get it right”

Bacteria at the beach: High levels found at nearly a dozen locations in past two weeks



READER

NEWS &POLITICS

NEWS & POLITICS | MUSIC | ARTS & CULTURE | FILM | FOOD & DRINK | CLASSIFIEDS

July 11, 2018

Lake surfers say polluted waves are making them sick—but they love it too much to stop

Surfers catch waves last month off the shore of Whiting, Indiana

RICHARD ANDERSON

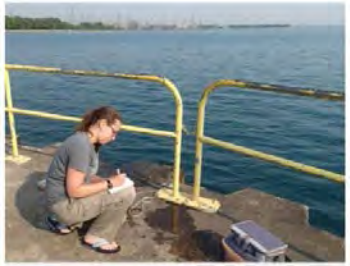
Cause of E. coli beach closings? Gulls

Pathogen presence diminishes Indiana beach water quality

Date: May 23, 2018

Source: American Society of Agronomy

Summary: Researchers have recently published results identifying the major sources of E. coli breakouts on several beaches on Lake Michigan. They have also researched an effective method of reducing the breakouts and the resulting beach closings.



Water samples were collected and analyzed for markers indicating the source of bacteria to beaches, and gulls were a significant source.

Credit: USGS

Letter: Dog-breeding business poses potential issues

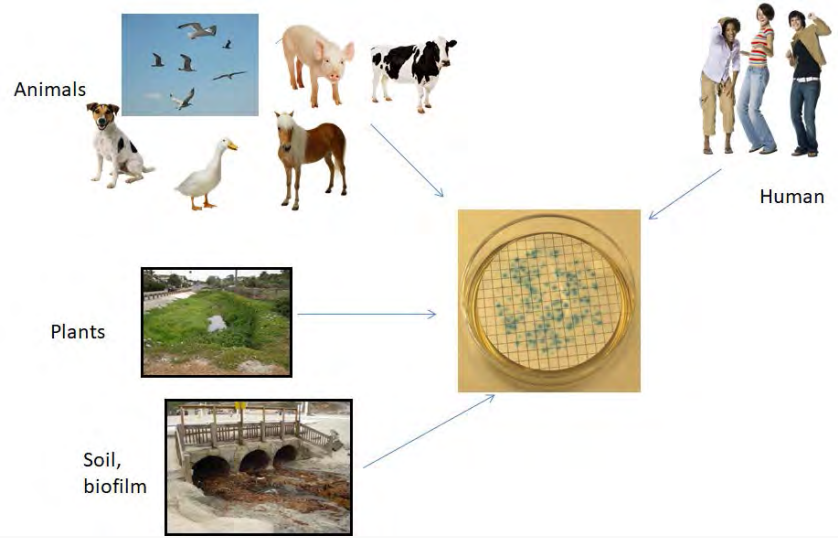
By Staff Reports - 1/19/18 11:39 PM

From: Nancy Ray

Columbus

Source Molecular

Available Tools - Legacy Testing (Culture FIB)



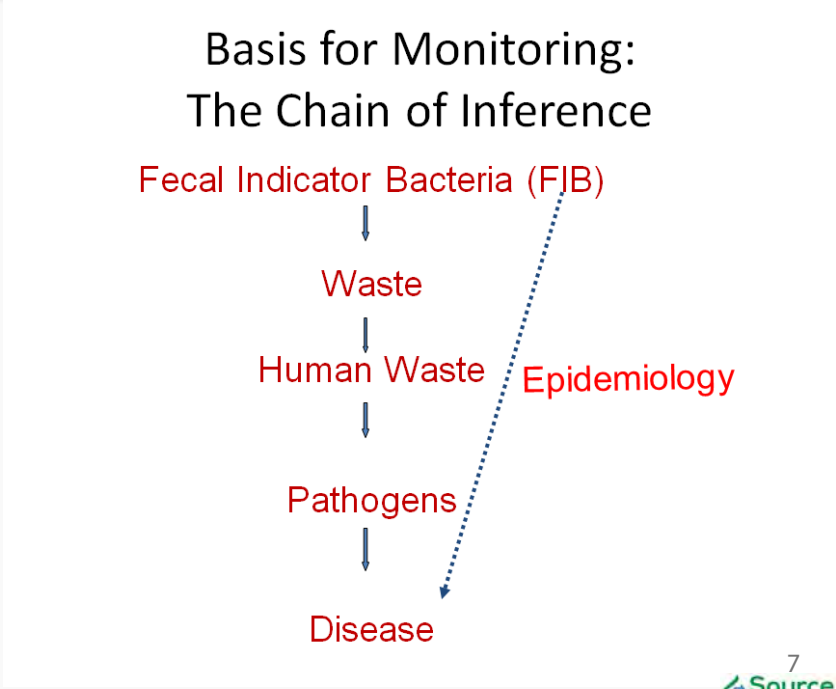
Concerns: Ineffective at discriminating between sources.

Available Tools - Field Observations

Concerns: Circumstantial and subjective evidence. Difficult to defend.

Consequence

- Hinders source abatement
  - Source identification must precede mitigation
- Weakens the chain of inference
  - Not all sources present the same level of human health risk
    - Non-fecal < fecal
    - Non-human < human



DNA-based Microbial Source Tracking

- There are special microbes that are only associated with a given source
  - Host and gut microbes co-evolve
    - Physiological difference of the gut
    - Dietary difference between hosts

- MST provides a set of methods to identify sources of contamination

Example Datasets: Questions Answered

- Monitoring Receiving Waters for Human
- Monitoring Outfall Discharges
- Monitoring Receiving Waters for Multiple Sources

Advance

Monitoring Receiving Waters – Locating Sources

- Aging infrastructure in urban watershed
- High concentrations of E. coli
- Human sources expected from leaking sewers
- Animal sources also suspected

What segment would you prioritize?

	Human-Associated DNA
Site	**Copies/100ml**
A	9480
B	105000
C	479
D	13100
E	Detected
Blank	Not Detected

Monitoring Receiving Waters – Locating Sources

- Bracketed drainage areas
- Upstream and Downstream comparisons

	Human-Associated DNA		
	Copies/100ml		
Site	**Upstream**	**Downstream**	**Delta**
A	20000	9480	decrease
B	Detected	105000	**increase**
C	1170	479	decrease
D	6750	13100	**increase**
E	Detected	Detected	none
Blank	Not Detected		NA

Menu

Monitoring Outfall Discharges – Locating Sources

- Combined Storm Sewer with know CSOs
- E. coli concentrations measured at outfalls

Location	All	Wet	Dry
A	25.18	224.15	38.15
B	23.30	54.87	17.11
C	20.21	66.54	24.76
D	82.88	125.28	26.60
E	52.14	169.15	20.34
F	10.20	8.15	77.58
G	310.23	310.23	NA
H	19.68	121.06	65.36

Which outfall would you prioritize?

Monitoring Outfall Discharges – Locating Sources

- Human-associated DNA measured simultaneously

Ranked on E. coli Concentrations			
Location	**All**	**Wet**	**Dry**
A	4	**2**	3
B	5	7	7
C	6	6	5
D	**2**	4	4
E	3	3	6
F	8	8	**1**
G	**1**	**1**	NA
H	7	5	**2**

Ranked on Human DNA Detections			
Location	**All**	**Wet**	**Dry**
A	7	4	6
B	5	7	**2**
C	4	5	3
D	3	3	4
E	**2**	**1**	**1**
F	8	8	7
G	**1**	**2**	NA
H	6	6	5

Which outfall would you prioritize?

Menu

Monitoring Receiving Waters for Multiple Sources

- 4 sampling events in 7 streams (June to October)

Stream	E. coli Exceedances	Goose	Dog	Human
A	3	0	4*	4
B	1	0	3	4*
C	3	0	4	4
D	3*	0	4	4
E	1	0	2	0
F	2	0	4	1
G	3	0	4	1
*Highest Concentration Measured				

What remediation would you propose?

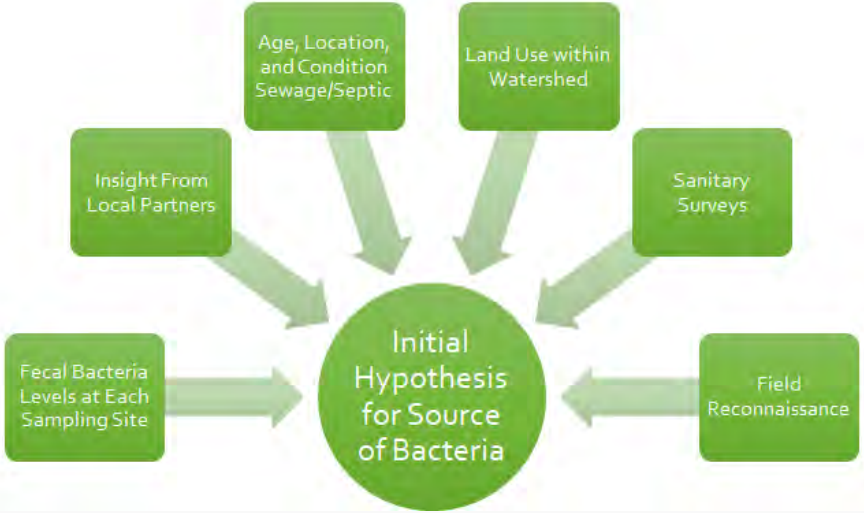
Menu

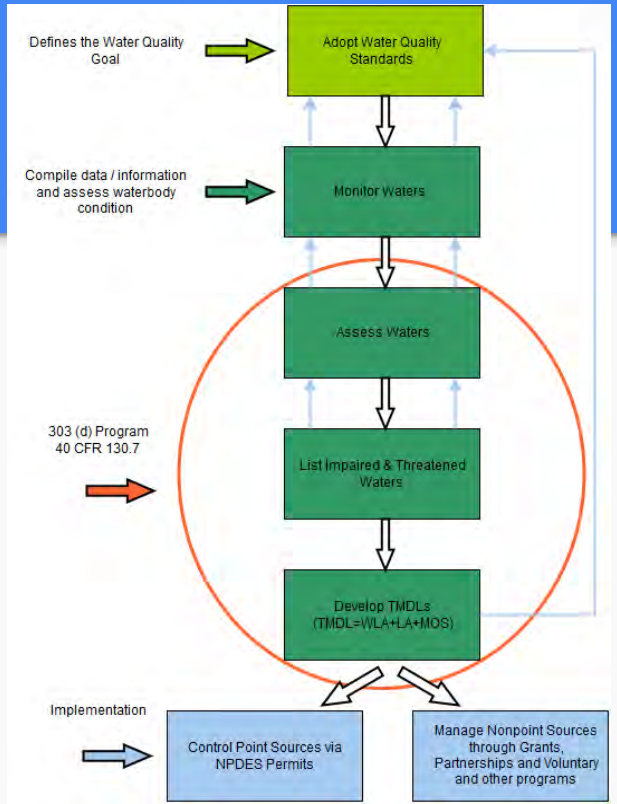
Designing a Project

MST is being applied at each stage of the CWA

Define Project Objectives

IDDE
Compliance Demonstration
Natural Source Exclusion
Site Specific Objectives
Infrastructure Asset Management





Sampling and Testing Plan

Sampling Sites

- Fecal Bacteria Hotspots
- Collecting Near Physical Sources
- Represent Watershed's Spatial Variability

Sampling Events

- Wet/Dry Weather Sampling
- Seasonal Changes
- Significant Number of Events to Represent Temporal Variability

Tests Per Sample

- Focus on Anthropogenic Sources (Human, Dog, Agriculture)
- Most Likely Wildlife Source (Birds, Deer, ect)

~$250-$800/sample

Case Studies: Outcomes Achieved

- MS4 IDDE Evaluation
- BMP/Remediation Planning
- BMP Effectiveness Monitoring
- Natural Source Exclusion

Advance

Where is the pollution coming from - Boston Water and Sewer

- First ever effectiveness assessment of MS4 IDDE program using DNA markers
- Human markers measured at outfalls regardless of degree of IDDE completion, and conventional tools (test kits) found to be insufficiently sensitive or specific for detecting illicit discharges
- New IDDE procedures now recommended, including DNA markers to improve program effectiveness
- Outcome will be greater bacteria and phosphorus reduction (at **lower unit cost and greater health benefit** than Green Infrastructure), **moving City closer to TMDL compliance**
- Project recognized with national O&M Performance Award from NACWA

Geosyntec consultants

NACWA NATIONAL Environmental Achievement AWARDS

Menu

Source Molecular

Site Prioritization - Florida DEP/Martin County

Summary of Costs / Ranking (Total)

Ranking	Communities	# of Parcels	Totals Cost			Cost Per Parcel		
			Vacuum Collection System*	Gravity Sewer System*	Grinder Pump System**	Vacuum Collection System*	Gravity Sewer System*	Grinder Pump System**
1	Martin Downs / Sunset Gardens (Old Palm City) Area	1078	$13,532,368	$20,852,279	$16,412,734	$12,553	$19,343	$15,225
2	Golden Gate Subdivision	775	$9,580,163	$15,656,572	$11,923,603	$12,373	$20,202	$15,385
3	New Rivage Subdivision	256	$3,974,236	$5,909,109	$4,029,218	$15,524	$23,082	$15,739
4	Transit Ave Area	277	$4,445,731	$6,817,429	$4,447,102	$16,050	$24,612	$16,055
5	Hibiscus Park Area	1349	$14,165,566	$15,617,161	$18,924,589	$10,501	$13,891	$14,029
6	Port Salerno / New Monrovia Area	878	$10,536,133	$14,685,435	$12,878,941	$12,000	$16,719	$14,668
7	Salerno / Manatee Pocket Area	478	$8,434,992	$7,858,844	$6,905,908	$11,370	$16,441	$14,448
8	Not							$16,802
9	Rive							$15,452
10	Sout							$23,178
11	Sout							$15,398
12	Town of Sewall's Point	931	$11,559,281	$16,658,259	$14,024,305	$12,416	$17,893	$15,064
13	Rio / St. Lucie (East)	831	$4,223,304	$6,408,478	$4,894,485	$12,759	$19,361	$14,787
14	Boardwalk / Balloon Bay	25	--	$1,178,085	$437,729	--	$46,090	$17,509
15	Stuart Yacht & Country Club	504	$7,065,980	$9,940,480	$7,596,345	$14,020	$19,064	$15,072
16	Four Rivers Subdivision	106	--	$3,171,304	$1,955,547	--	$29,919	$18,449
17	Crane Creek / Country Club	381	$6,402,014	$10,991,924	$6,385,546	$16,801	$28,849	$16,760
18	North Rivers Shore - Phase 2	202	$4,186,403	$6,168,554	$3,484,891	$14,337	$21,125	$15,399
19	Tropical Farms Area	652	$9,846,995	$14,296,887	$10,299,471	$15,102	$22,004	$15,797
20	Rivers End Subdivision	113	--	$3,050,687	$2,011,467	--	$26,997	$17,801
21	Vista Salerno / US 1 Area	234	$3,404,083	$4,781,277	$3,558,041	$14,547	$20,433	$15,205
22	Rio / St. Lucie (West)	97	--	$2,197,773	$1,582,174	--	$22,657	$16,311
23	Captain's Creek Subdivision	167	--	$4,591,743	$2,769,291	--	$27,495	$16,583
24	Lake Grove Subdivision	76	--	$2,027,883	$1,395,236	--	$26,683	$18,358
	Totals***	10,388	$118,092,117	--	$20,150,037			
			Total Cost****		$138,232,154			

Total Cost** $138,232,154

Ranking Summary

Ranking	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Communities	a Downs / Sunset ns (Old Palm City)	n Gate	Rivage	s Ave	an Park	alerno / Monrovia	o / Manatee Pocket	Mapp Road	rson (G&CC (Hideaway Mid Rivers)	Mapp Road	Fork Area	l's Point	St. Lucie (East)	ralk / Galleon Bay	Yacht & Country Club	Rivers	Creek Country Club	Rivers Shore - PH 2	ral Farms	s End	Salerno / US 1 Area	St. Lucie (West)	in's Creek	Lake Grove
Human Biological Markers									12			12			0			0				0		
Total Score									99.35			90.85			85.51			84.37				82.93		
Populat Density																								4
Potable Systems																								12
Classifi Surface																								4
Proxim Surface																								4
FEMA Plain																								4
Ground Table																								8.85
Soil Typ																								12.00
Surface Water Management	12	12	12	12	12	12	12	12	10	12	4	12	12	8	12	4	12	12	9	4	12	12	4	4
Nitrogen Contribution	9.59	6.89	2.28	2.46	12.0	7.81	4.23	1.46	7.87	0.33	2.42	8.28	2.98	0.22	4.48	0.94	3.39	2.60	5.80	1.01	2.08	0.86	1.49	0.68
Human Biological Markers	12	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Score	99.35	90.85	85.51	84.37	82.93	82.30	81.90	80.66	79.64	79.36	76.33	74.57	73.98	73.15	72.69	72.10	71.65	71.35	71.17	70.80	68.62	68.52	65.74	53.53

Menu

Demonstrating BMP effectiveness - Santa Barbara

Arroyo Burro Beach Source Tracking Project

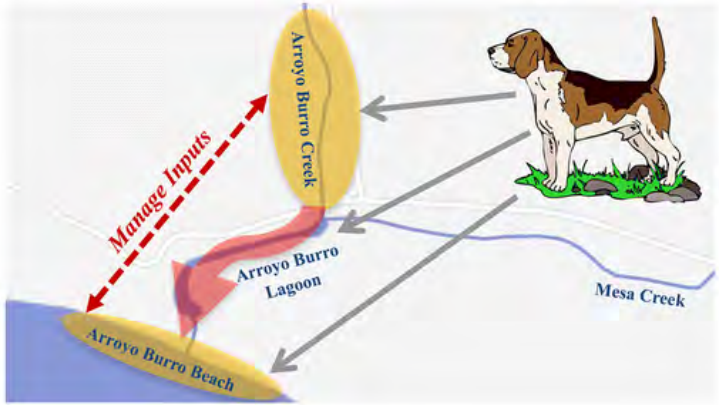
Multiple Source Markers to test hypotheses

Scenario

- High Fecal Bacteria
- Potential Sources:
  - Human
  - Horse
  - Gull
  - Dog

http://www.independent.com/news/2007/dec/01/arroyo-burro-beach/

Microbial Source Tracking in a Coastal California Watershed Reveals Canines as Controllable Sources of Fecal Contamination
Jared S. Ervin et al. 2014



Outcomes

- Horse not detected
- Gull confirmed at lagoon and beach
- Dog markers reduced after targeted public outreach
- Human markers associated with homeless encampments

Natural Source Exclusion – central Florida

Water Research
Volume 144, 1 November 2018, Pages 424-434

Determination of wild animal sources of fecal indicator bacteria by microbial source tracking (MST) influences regulatory decisions

K.H. Nguyen, C. Senay, S. Young, B. Nayak, A. Lobos, J. Conrad, V.J. Harwood

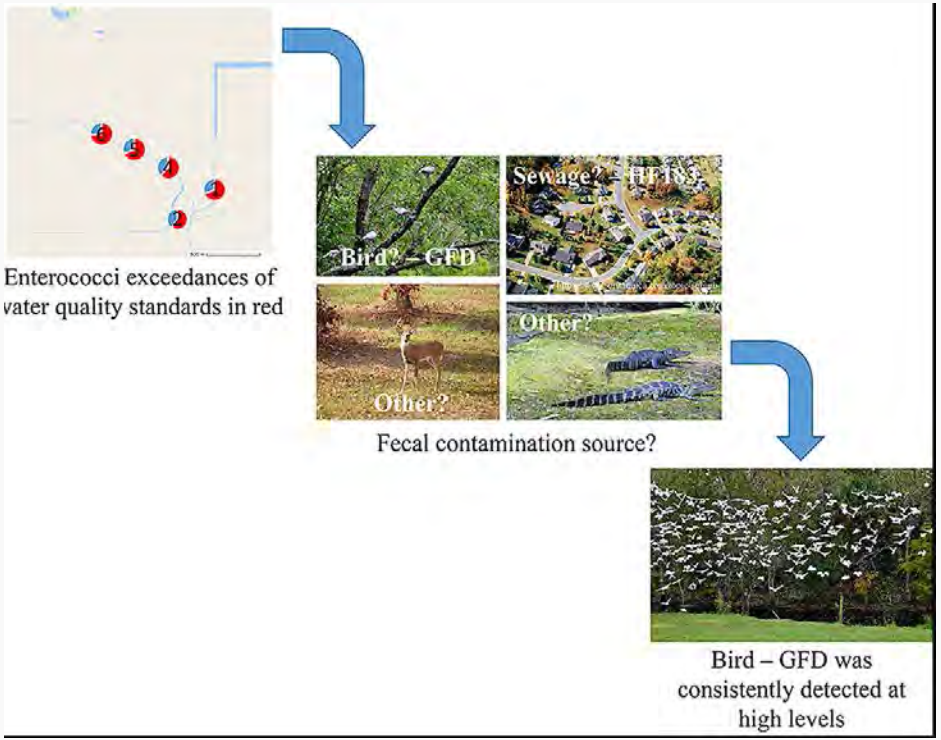
Show more

https://doi.org/10.1016/j.watres.2018.07.034

Get rights and content

Highlights

- High FIB led to suspicion of sewage contamination in a stream managed for wildlife.
- Predictive modeling identified major factors influencing FIB levels e.g. seasons.
- Bird marker gene (GFD) levels were high throughout the year.
- Sewage markers (HF183) were attributed to cross reaction with deer feces.
- Natural bird sources contributed to FIB contamination in the water body.



- Water, sediment and vegetation samples
- Fecal validation samples
- Stream removed from 303d

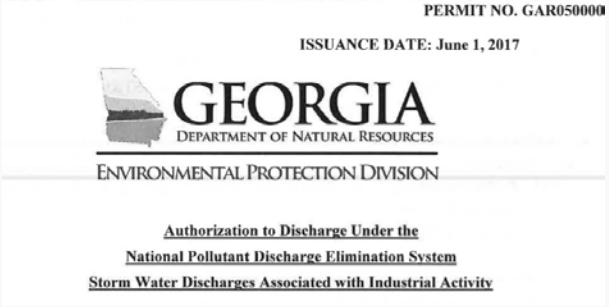
Menu

Microbial Source Tracking - Maturity

Precedent	✔	Projects in >40 States
Credible Tests	✔	National Validation (SIPP)
Access to Technology	✔	Laboratories (Accredited)
Objective Interpretation	✔	Host Fecal Score

MST's in stormwater permitting

GA NPDES Industrial Storm Water General Permits



PERMIT NO. GAR050000

ISSUANCE DATE: June 1, 2017

GEORGIA
DEPARTMENT OF NATURAL RESOURCES
ENVIRONMENTAL PROTECTION DIVISION

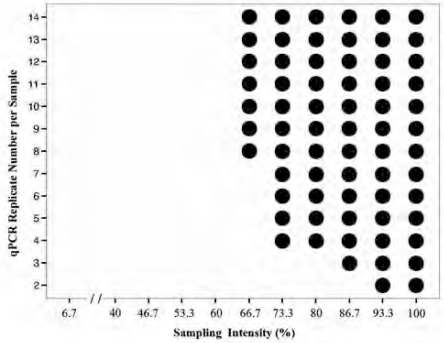
Authorization to Discharge Under the
National Pollutant Discharge Elimination System
Storm Water Discharges Associated with Industrial Activity

C.2.4.2 Scientific testing, such as DNA analysis, may be used to document that bacteriological constituents found in stormwater discharges from the facility are not present as a result of industrial activity at the site or are below the impaired waters benchmark for fecal coliform. Permittees must submit the testing program to EPD and obtain approval prior to conducting the testing. The results of the testing must demonstrate that bacterial contamination from industrial activity does not contribute to a violation of water quality standards.

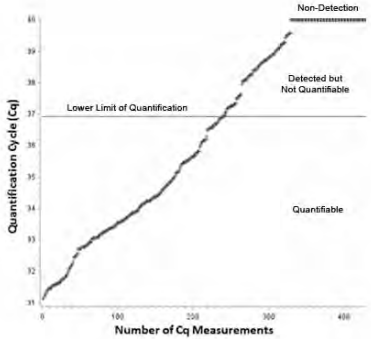
Project Level Probabilistic Modeling

HUMAN FECAL SCORE FOR SITE RANKING

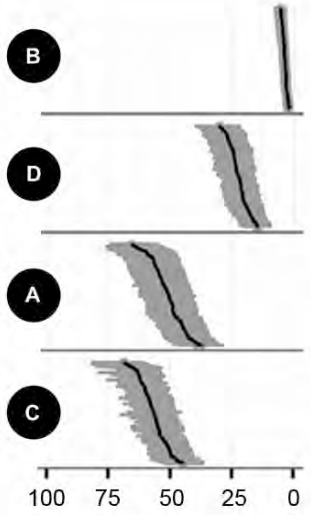
Polluted Sites



Customized Field Sampling and qPCR Solutions



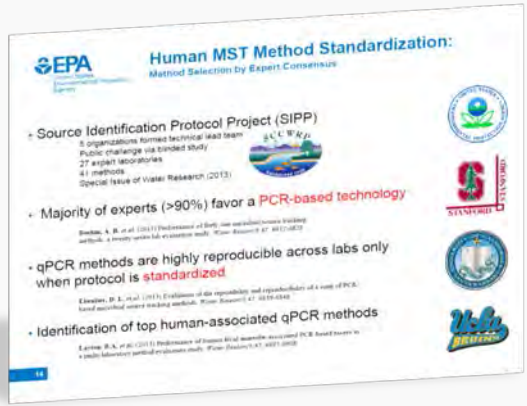
All qPCR Data Included



Human Fecal Score
(Copies per 100mL 95% BCI)

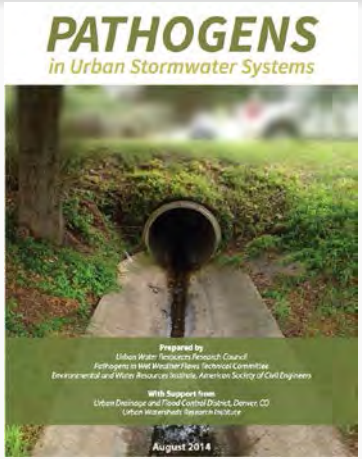
STANDARDIZED PROCEDURE

Microbial Source Tracking Resources



Source: Orin Shanks, Biological and Microbial Aspects of Septic System Pollution webinar, June 30, 2015

California Microbial Source Identification Manual



Report on state-of-the-practice on source tracking techniques and strategies

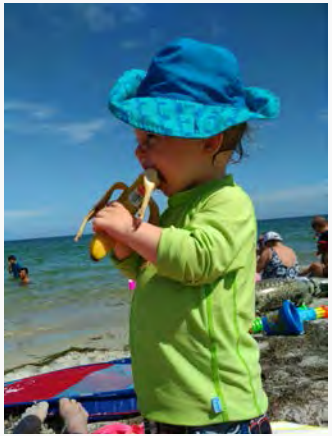
Thank You

Source Molecular

ACCREDITED
Certificate 4126.01
ISO 17025 Accredited
Testing Laboratory

www.sourcemolecular.com

James Herrin
Project Manager
jherrin@sourcemolecular.com
786-220-0379



QUIT

Question?
Uninterested at this time
Information, please.
Time to Talk!

Next Steps - Automation

https://www.youtube.com/watch?v=i_2Q1DFLp3g