



Developments in Microbial Source Tracking

Digital PCR, California's Source ID Manual, and US EPA tests



Experience

- Leading commercial practitioner of Microbial Source Tracking services
- >70 Source Tracking Studies Completed in 2014
- Founded in 2002

Research

- Commercial partner for California Source Identification Project (SIPP)
- Participated in US EPA Method Standardization Study
- Collaboration with SCCWRP on Digital PCR study (publication pending)

Methods

- US EPA-Developed
- Cattle
- Human
- Chicken
- Dog

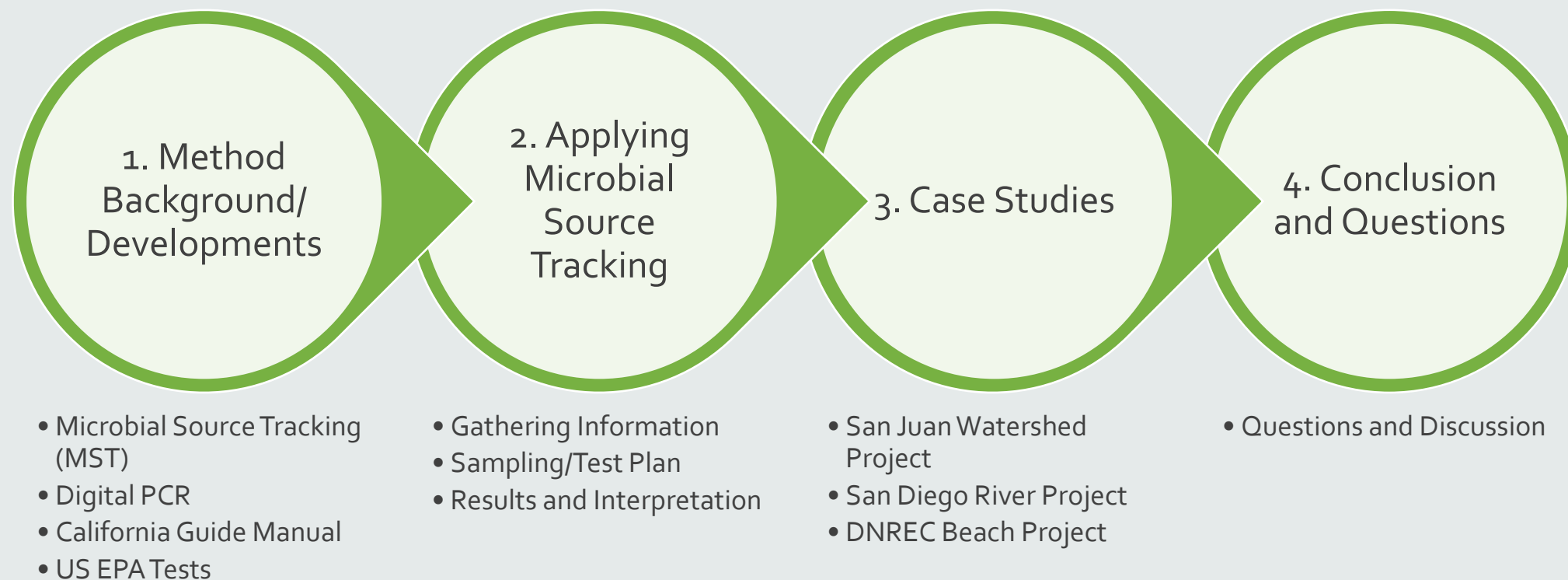
- 9 additional hosts available

Quality

- Pending ISO 17025 laboratory accreditation

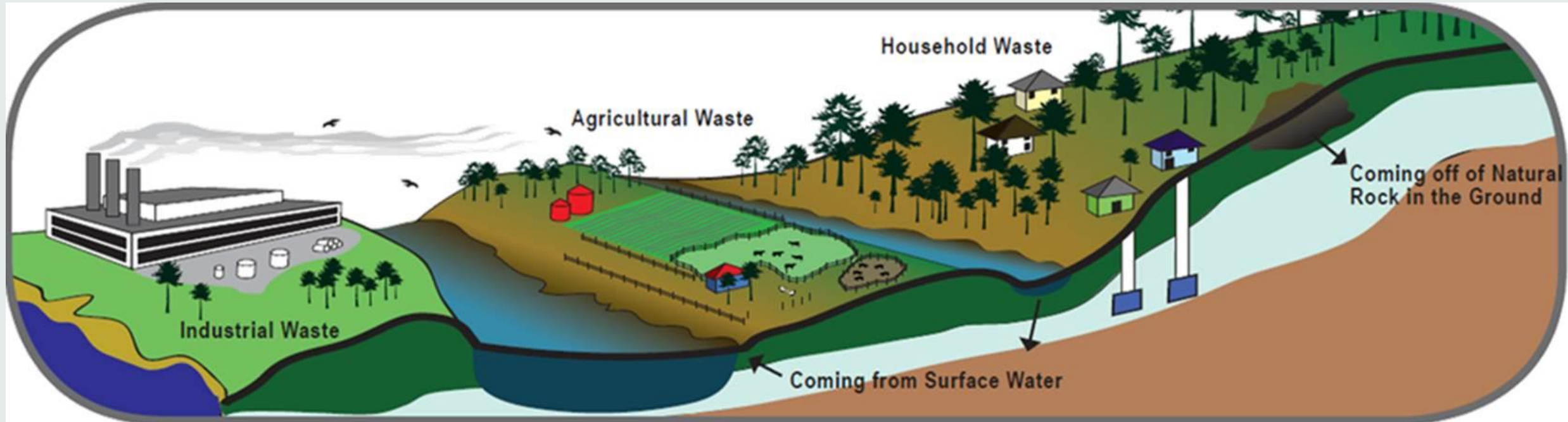


Presentation Overview



Problem

Fecal pollution entering the watershed



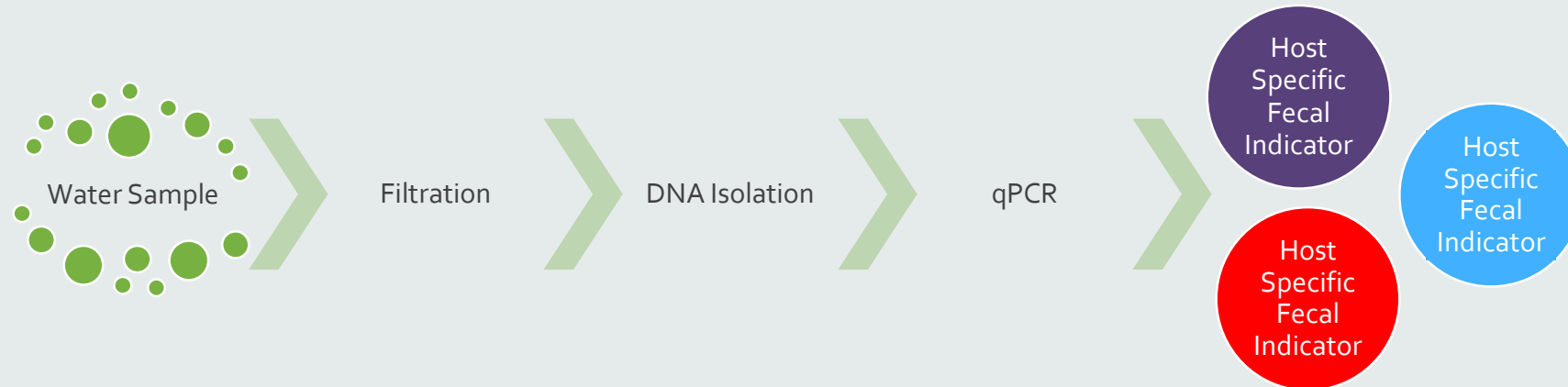
(NC Department of Health and Human Services, 2011)

Method Background

Traditional Fecal Indicator Methods



Microbial Source Tracking Methods



Developments

Digital PCR

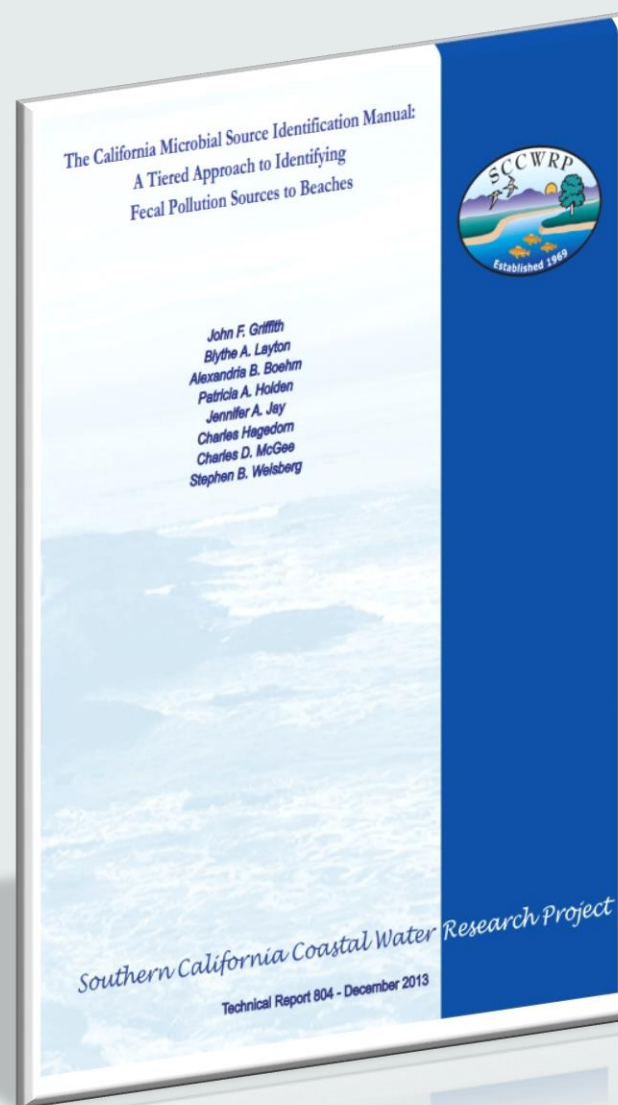


<http://youtu.be/S5SvaPwxXiU>

Study Link: <http://www.sciencedirect.com/science/article/pii/S0043135414008409>

Developments

California Microbial Source Identification Manual



- [Link to Document](#)
- Created by SCCWRP for CA Water Resource Control Board
- Tiered Approach to Identifying Bacteria Sources
- Scientific Consensus! 27 Participating Labs (including Source Molecular)
- Set precedent for Microbial Source Tracking to be used as a mainstream tool in California

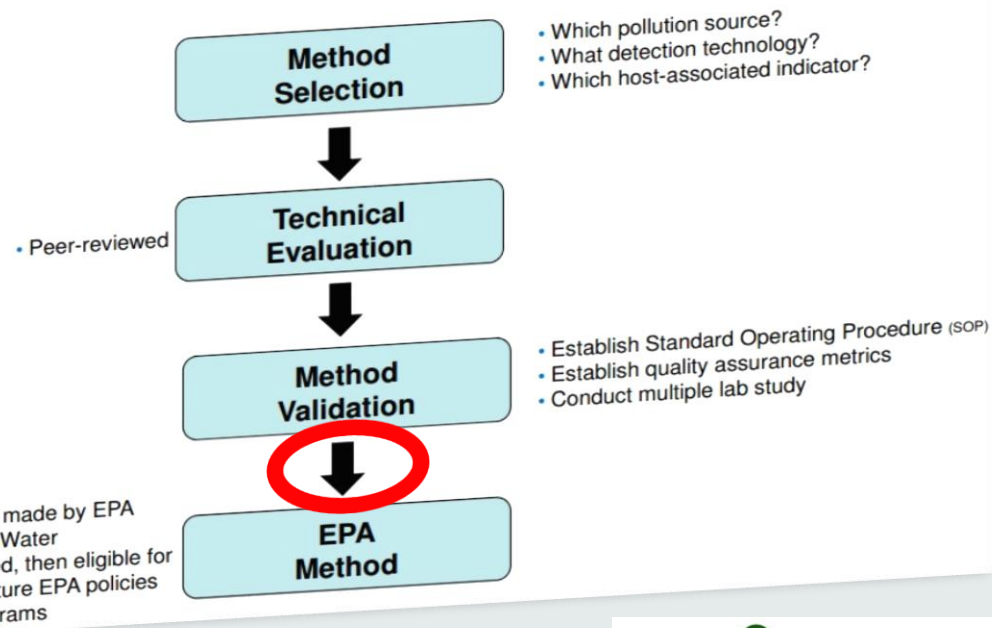
Developments

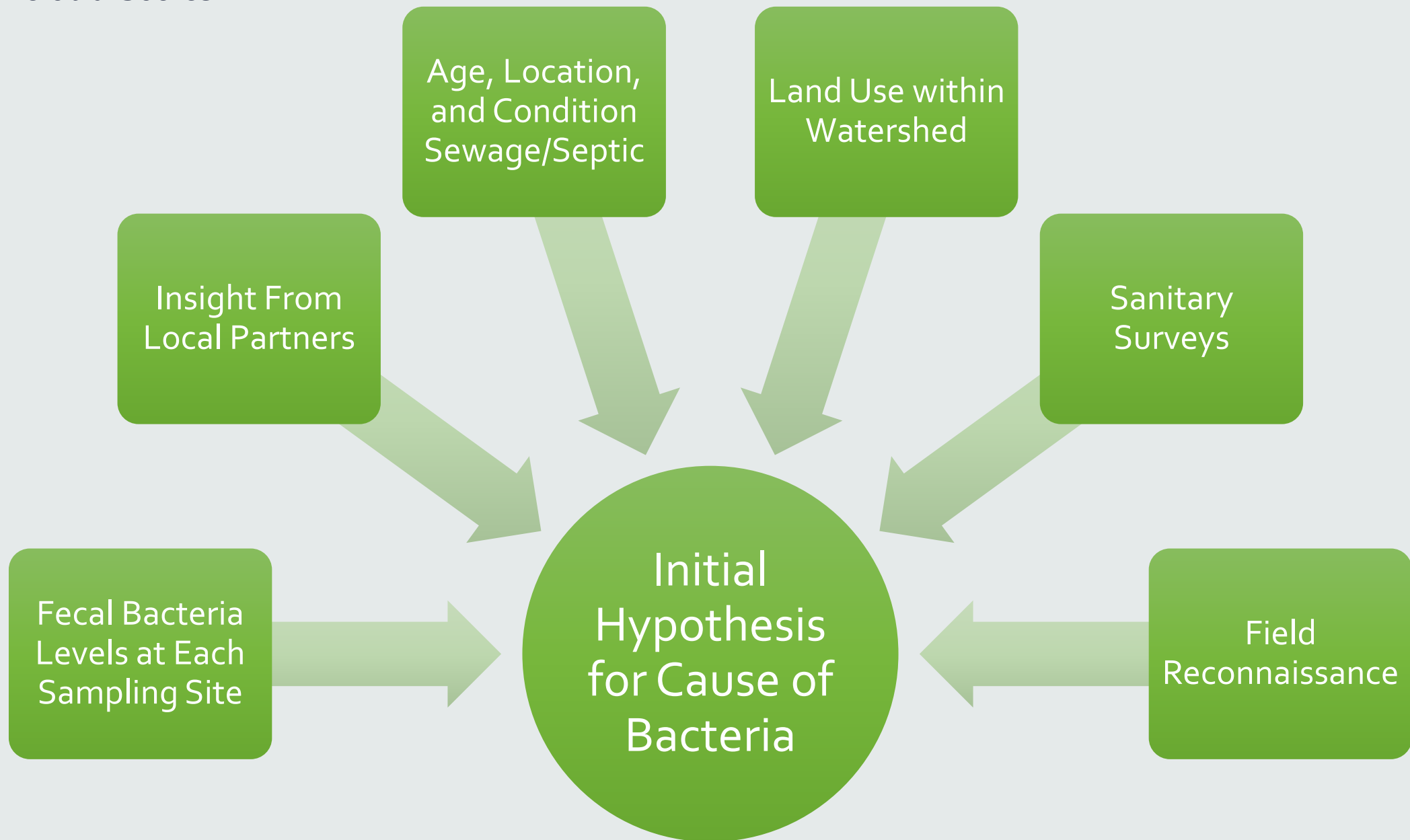
US EPA Developed/Patented Tests



Upcoming US EPA Approved Method

EPA Method Standardization: Development Plan





Sample and Test Plan

- Fecal Bacteria Hotspots
- Collecting Near Physical Sources

Sampling Sites



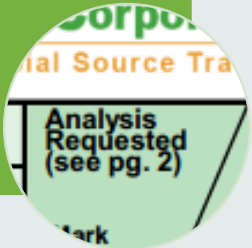
- Wet/Dry Weather Sampling
- Seasonal Changes
- Statistically Significant Number of Events

Sampling Events



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

Tests Per Sample



Submitting Samples



Pre-Filtered
Samples



Ship Water
Samples



Samples
Shipped
Overnight

Results

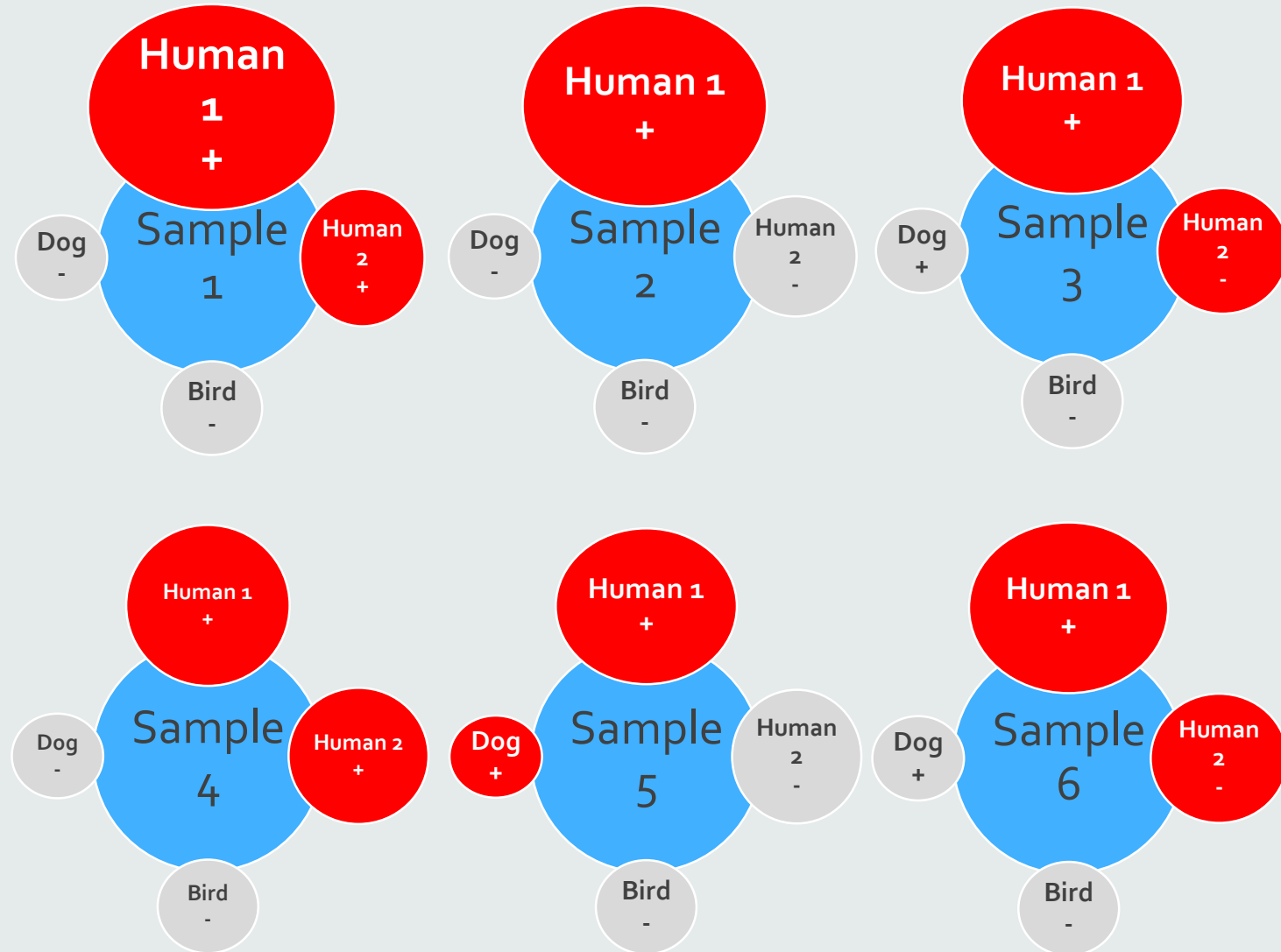
Example Results for Site 1 6 Sampling Events

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 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

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 #	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



Interpreting Results

Scenario 1

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

Non-Human
Pollution
Source

Human Sources

Fecal Indicators Present Only
During Wet Weather

Presence of Gull Fecal
Indicators

High Concentrations of Gull
Fecal Indicators

Sanity Surveys show systemic
presence of Gulls on the
beach

Absence of Human Fecal
Indicators

Sanity Surveys Showed No
Evidence of Leaking Sewers

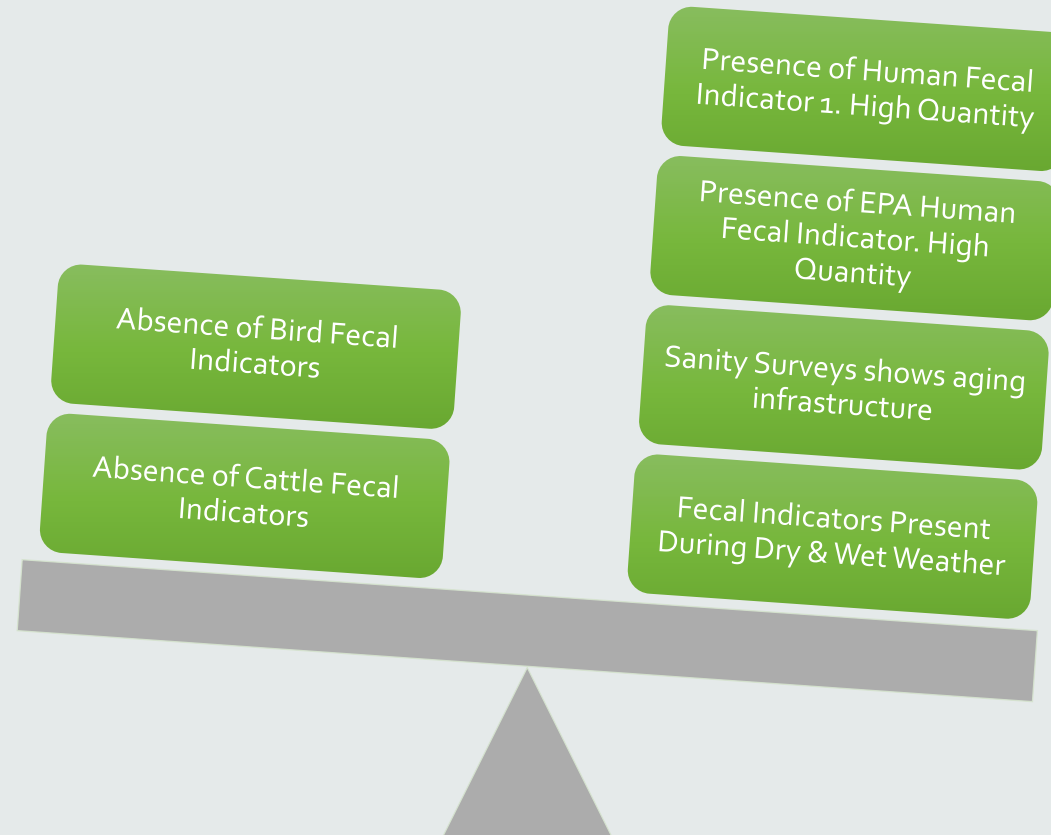
Interpreting Results

Scenario 2

- High Fecal Bacteria
- Potential Sources:
 - Human
 - Bird
 - Cattle

Non-Human
Pollution Source

Human Sources



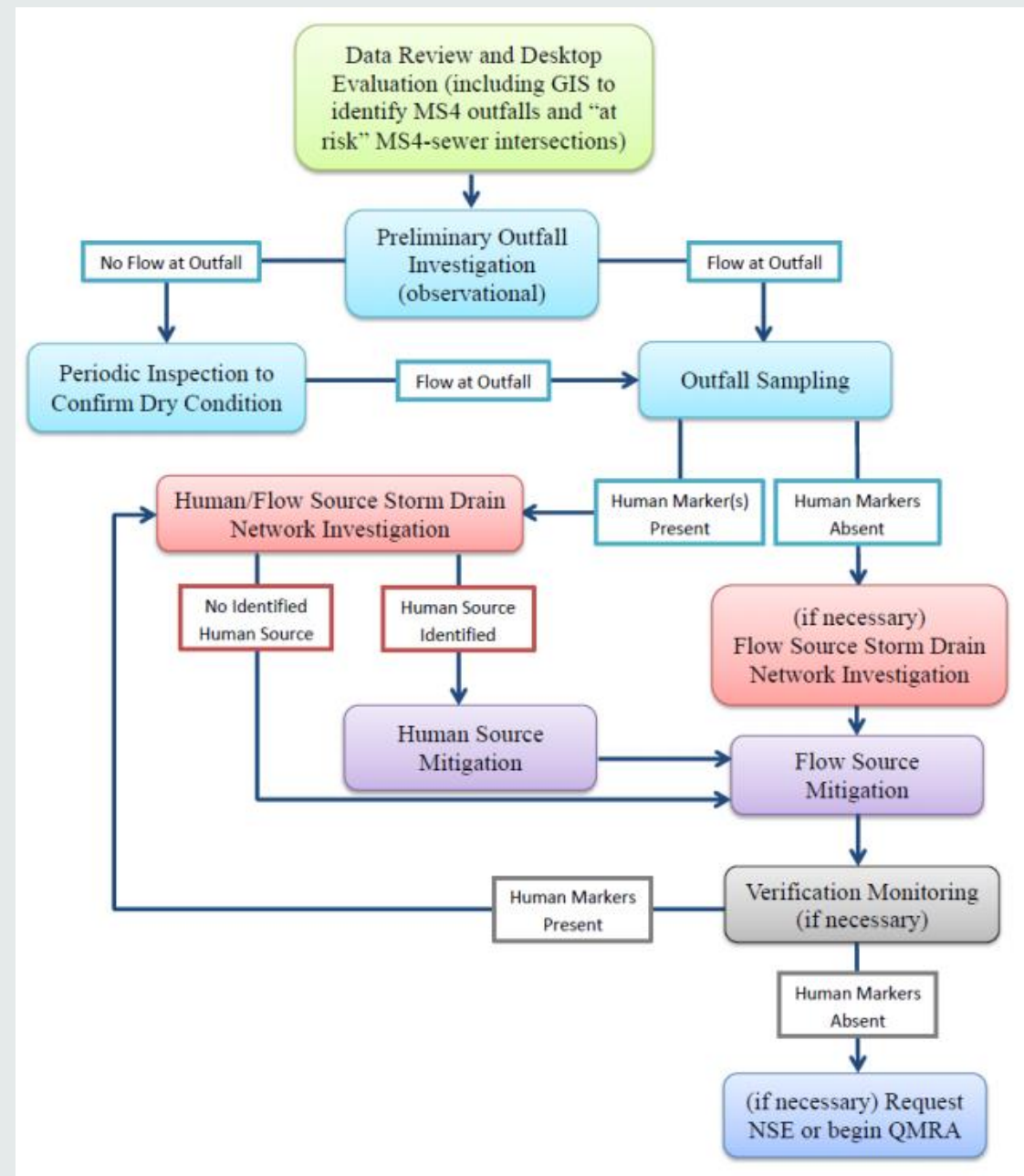
San Juan Watershed Case Study



<http://www.kob.com/article/11687/?vid=5035312&v=1>

Case Study 2

San Diego River MS₄ Project



[LINK: Pathogens in Urban Stormwater Systems](#)

Figure 5-4. MS₄ Microbial Source Identification Investigation Approach
(Source: Brandon Steets, Geosyntec Consultants)

Case Study 3

DNREC Beach Source Tracking Project

Problem

- high levels of Enterococcus at an estuarine inland recreational beach.

Existing Evidence

- Dog: Visual observation of dogs at the beach
- Gull: Often large concentrations of gull.
- Human: Geographic proximity to septic systems.

Hypothesis

- Gull, Dog, or Human are likely sources of high bacteria.

Sampling Plan

- Bacteria levels tested on each sample
- 3 sample locations (composited)
- 3 sampling events per week for ~3 months
- 49 samples total

Case Study 3

DNREC Beach Source Tracking Project

Test Plan

- 2 tests for Human
- 1 test for Dog
- 1 test for Gull

Submission

- Samples filtered locally. Shipped on dry ice.

Results

- **Human**
 - 92% of samples **negative** (N=49)
 - Quantification – low levels
- **Dog**
 - 96% of samples **negative** (N=49)
 - Quantification - low levels
- **Gull**
 - 100% of samples **positive** (N=48)
 - Quantification - abundant levels

Interpretation

- Based on the existing lines of evidence and the genetic qPCR results gulls were likely the major contributor to the elevated levels of Enterococcus (Delaware's water quality indicator) at this beach.

Questions



4985 SW 74th Court
Miami, Florida 33155
Telephone: (786) 220-0379

Presenter contact information:
Mauricio Larenas
Email:
mlarenas@sourcemolecular.com

Organizer contact information:
Grace Anderson
Email:
ganderson@sourcemolecular.com
Direct Phone Number: 786-220-4654

