California Water Quality Monitoring Council

My Water Quality

A COLLABORATION BETWEEN THE CALIFORNIA ENVIRONMENTAL PROTECTION AND NATURAL RESOURCES AGENCIES

QMRA Eligibility: Microbial Source Tracking and Pathogen Testing

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The California Water Quality Monitoring Collaboration Network

Swimmable California Webinar Series

March 21, 2018

Source Molecular

ISO 17025 Accredited Testing Laboratory

Source Molecular

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

Shifts in regulatory focus

- 2012 RWQC: shift from fixed numeric standards to a risk-based framework

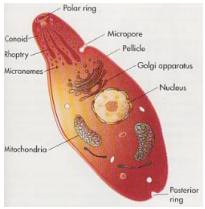
Assume: Indication of human fecal contamination

Focus: Public health risk

If FIB sources your site are mostly non-human or non-fecal, EPA allows establishment of site-specific criteria based on quantitative microbial risk assessment (QMRA).

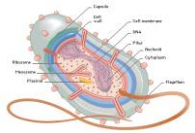
Fecal contamination

- What: Common pathogens in water
  - Protozoan pathogen
  - Bacterial pathogen
  - Viruses



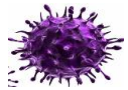
Protozoa

- Why: Public health risk limits beneficial uses
  - Agriculture
  - Drinking
  - Aquaculture
  - Recreation



Bacteria

Driver: Public health protection

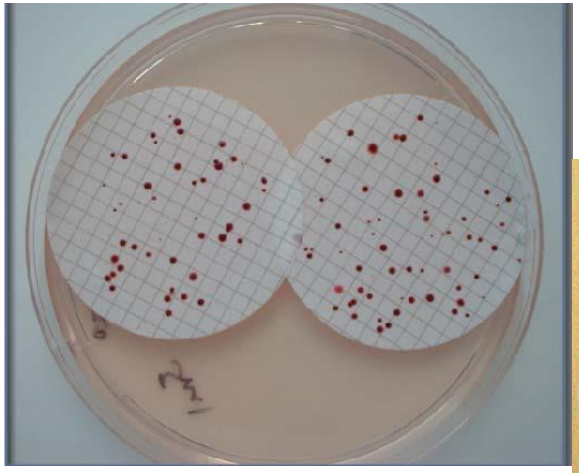


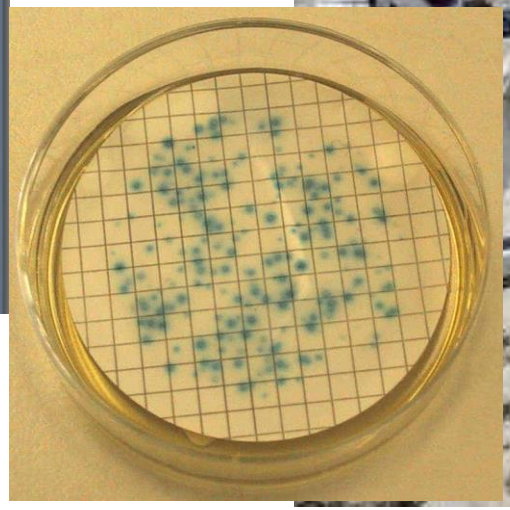
Virus

Source: google image

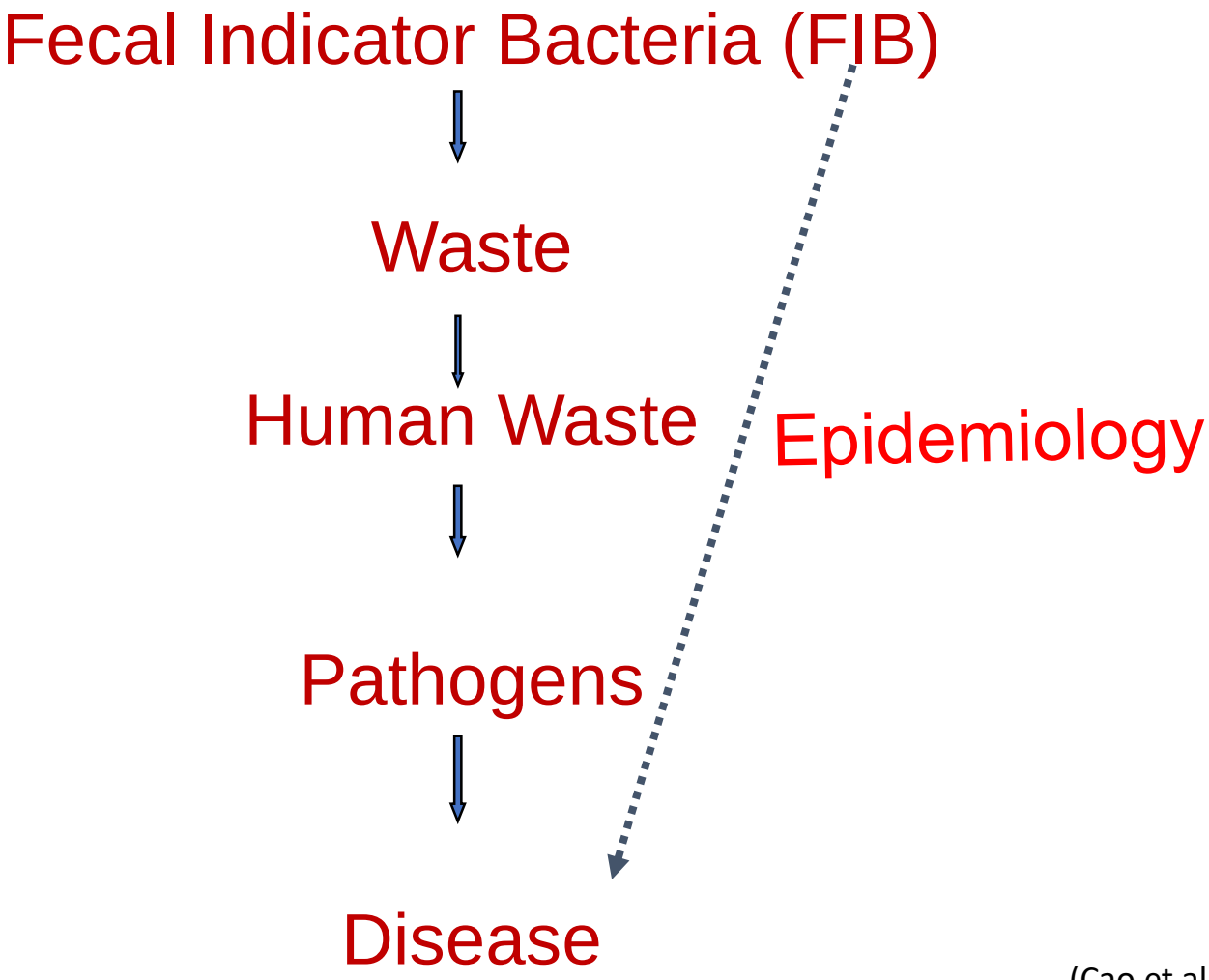
Recreational water quality monitoring

- Fecal Indicator Bacteria (FIB)
  - *Enterococcus* spp.
  - Fecal or total coliforms, *E. coli*





Basis for monitoring:
the chain of inference



(Cao et al 2010)

Water quality criteria

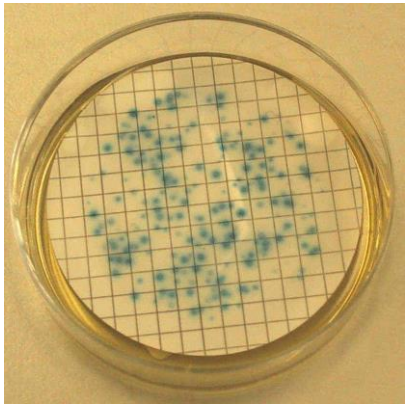
- Epidemiology studies established relationship between FIB concentration and public health risk
  - conducted in waters predominantly impacted by human fecal contamination

Criteria Elements	Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators	
	Magnitude	
Indicator	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]
Enterococci – marine and fresh	35	130
OR		
E. coli – fresh	126	410

Sites differ in sources

Animals

Human



Plants

Soil,
biofilm

Sites differ in sources

Animals

Fecal Indicator Bacteria (FIB)

Waste

Human Waste

Pathogens

Disease

Epidemiology

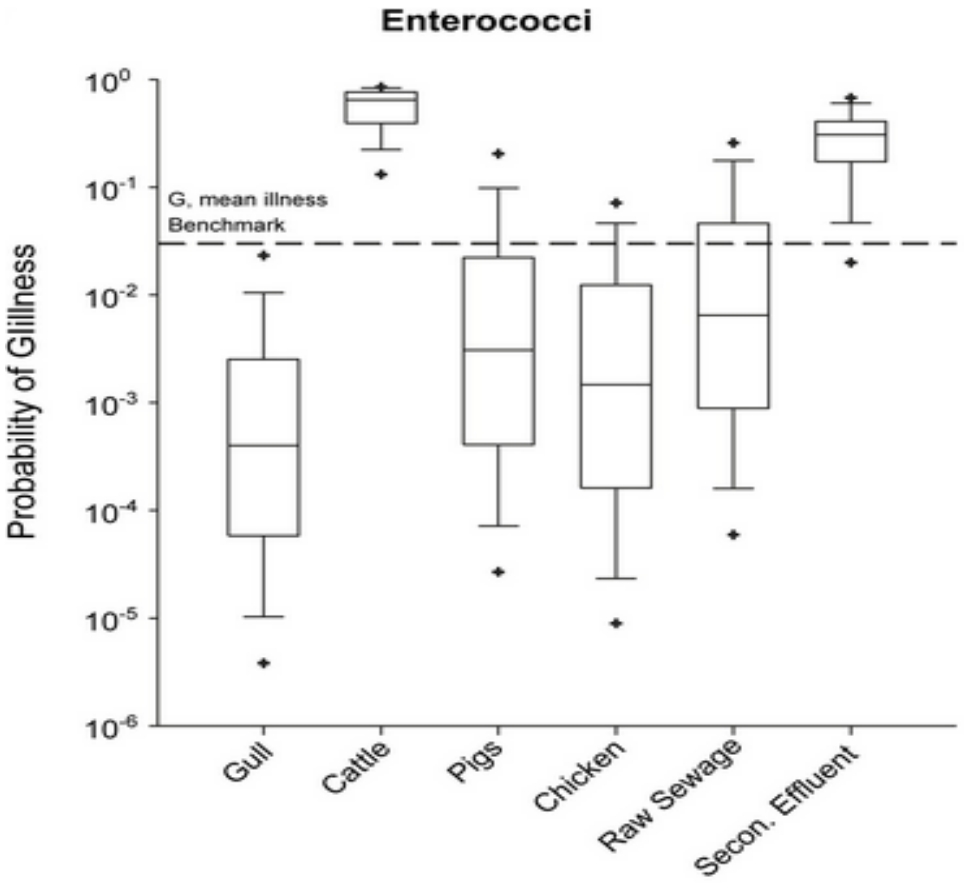
Human

Plants

Soil, biofilm

Sources differ in risk

- Same FIB concentration from different sources correspond to different level of human health risk



Soller et al. 2010

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Soller et al. 2010

QMRA concept

- Demonstrate human contribution is minimal
- Characterize water quality
  - Characterize sources at your sites → measure relevant pathogens that may infect human
- Modeling
  - Determine alternative site-specific criteria corresponding to the allowed risk in 2012 RWQC

Burden of proof

- Prove sources at your site are predominantly non-human, i.e. prove human fecal contribution at your site is minimal
  - In a scientifically sound and statistically defensible manner

- Currently, there is no guidance or standard procedure on how to do this

This presentation

- A scientific thought process on how to demonstrate human fecal contribution at your site is minimal
  - Not a policy talk
- Adapted from a guidance document submitted to LA regional board in 2016
  - Collective scientific understanding
  - New scientific advances since

Key areas of consideration

- What is considered “minimal human contribution”?
  - Is your site “non-human enough”?
- How do you measure human fecal contamination?
  - What marker? What technology?
- Sampling design considerations
  - Would 10 samples be enough? 10 samples from the same day at the same location?
- Lab and data analysis
  - Standardized protocols, quality

Non-human enough?

- Issue 1: No threshold

- USEPA has speculated using 10 - 20% as the threshold?
  - "Simulation shows that 10% - 30% human derived enterococci could serve as a potential threshold below which risk is assumed to differ substantially from risk from exposure to pollution that is 100% human." (Soller et al 2014)
  - Ultimately, this is a policy decision involving more than just scientific input.
  - As an example, let's assume using a 10% human contribution as the threshold.

Example: 10% of what?

- Issue 2: Interpretation of the threshold
- Ideal: less than 10% of the culturable enterococci originated from human fecal material
- Reality: We can't do culturable enterococci source apportionment.
  - Human fecal contamination is measured by non-enterococci genetic markers

Interpretation – by magnitude

- less than 10% of the “amount of fecal contamination” originates from human sources
  - total mass of fecal material, total fecal DNA, and total enterococci (by molecular method)?
  - Feasibility and appropriateness uncertain

- May be unattainable at the current stage

Interpretation – by frequency

- less than 10% of a certain “number of samples” are positive for human fecal contamination

- Defining number of samples
  - Total samples taken at the site
  - Number of samples exceeding enterococci criteria

- Definition of “positive”
  - Amplified, above limit of quantification
  - Moving target?

Interpretation – by benchmark against reference site

- Human fecal score (HFS)
  - A standardized mathematically defined formula to consistently quantify extent of human fecal contamination at a site
  - Integrates frequency and magnitude

- HFS at your site < HFS at a reference site

“Non-human enough” - sum

- Ultimately a policy decision based on the best available science
  - And the best data from the site under evaluation
- Stakeholders take the initiative to obtain the best study design, data to enable regulator decision making
  - And provide options, multiple lines of evidence

Measure human fecal contamination - human marker choices

- Which marker to use?
  - HF183, HumM2, CrAssphage
  - Marker of choice: HF183

- How many markers to use?
  - Limited resource: One
  - Resources are better spent on improving other study design elements such as sample size

Measure human fecal contamination – molecular technology choices

	qPCR	Digital PCR
Availability	Widely available	In some labs
Accuracy	Maybe biased (reliability of reference material)	Gold standard
Definition of positive	Challenging	Straightforward
Robustness against inhibition	More frequent false negative for “dirty” samples	More robust again “dirty” samples
Repeatability	High	High
Reproducibility	Relatively high under tightly controlled conditions (in one lab, standardized protocol)	High

(Cao et al 2016, 2017)

Sampling design – spatial coverage

- Ultimately depends on the desired coverage of the "site" in the potential site-specific alternative criteria

- USEPA states that site-specific alternative criteria can be applied to a water body that shows uniform water quality

- Spatial variability at your site
  - High: more sampling stations
  - Low: fewer stations

Sampling design – sampling frequency and time of the day

- Once per day

- In the early morning

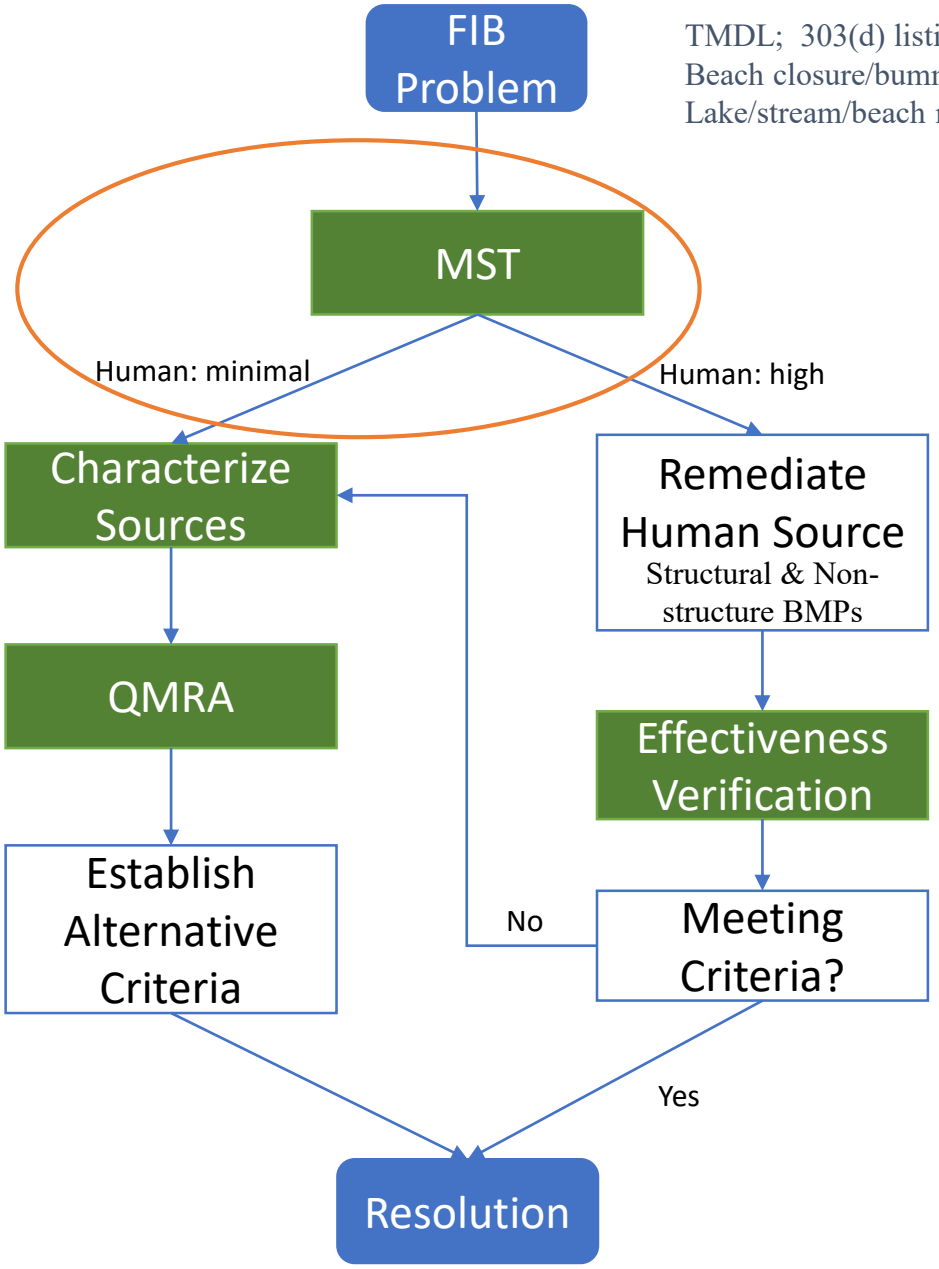
Sampling design – sample size

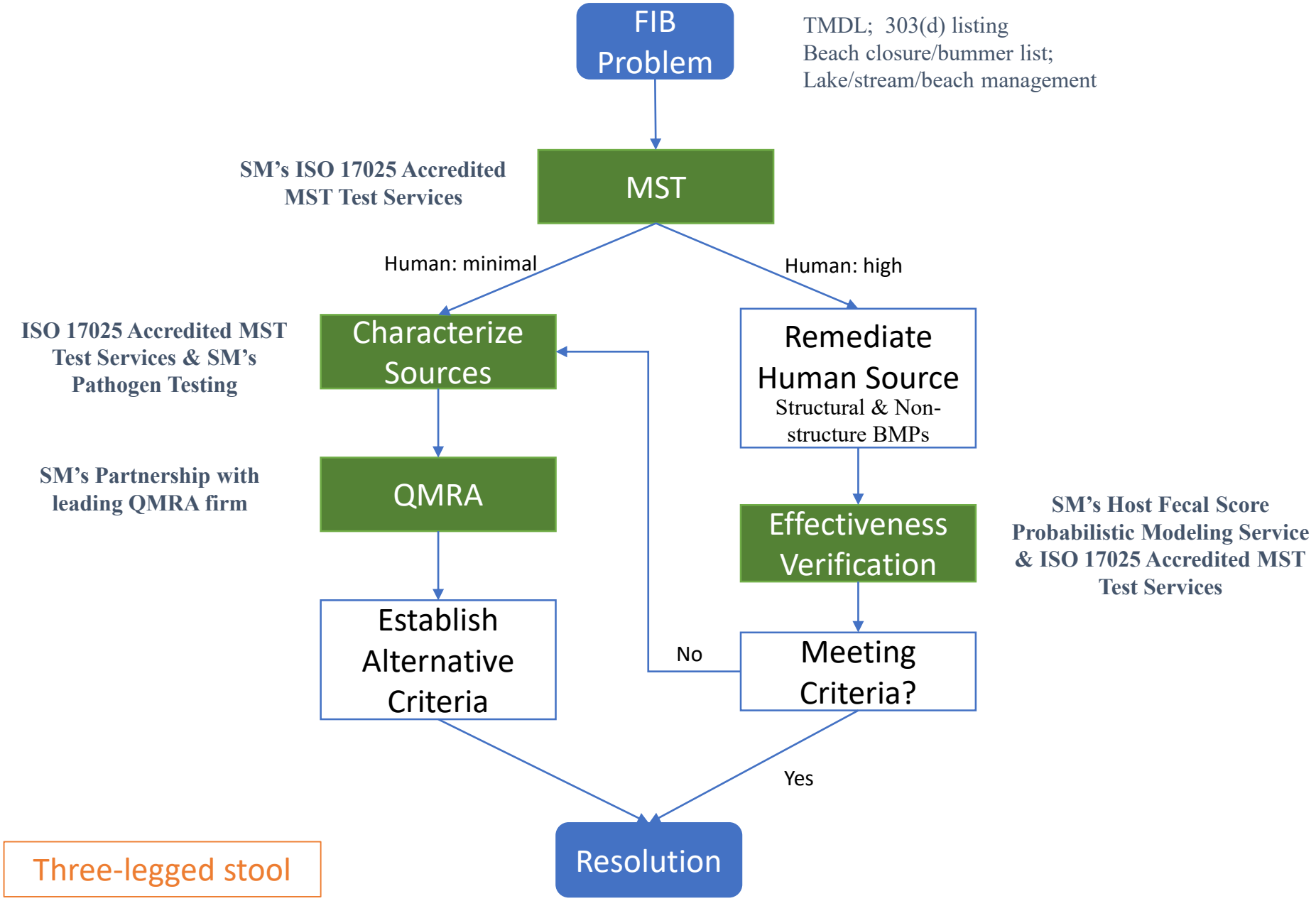
- Statistical power
  - Enough samples need to be collected to show
    - Example: frequency of HF183 positive is statistically significantly less than 10%
    - Example: Human fecal score is statistically significantly lower than that at a reference site
- Site temporal variability
  - Prior knowledge may not be available
  - 75% coverage is recommended
- Sample size vs. lab replication
  - Resource optimization: more samples

Lab and data analysis

- Standardized laboratory protocols must be followed to ensure consistent and high quality of the HF183 data.
- Standard protocols on data QA/QC should also be followed.
- Lab accreditation, automation, data acceptance criteria

(Shanks et al 2016)


FIB Problem
TMDL; 303(d) listing
Beach closure/bummer list;
Lake/stream/beach management
MST
Human: minimal
Human: high
Characterize Sources
Remediate Human Source
Structural & Non-structure BMPs
QMRA
Effectiveness Verification
Establish Alternative Criteria
No
Meeting Criteria?
Yes
Resolution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Characterize Sources
Remediate Human Source
Structural & Non-structure BMPs
SM's Partnership with leading QMRA firm
QMRA
Effectiveness Verification
SM's Host Fecal Score Probabilistic Modeling Service & ISO 17025 Accredited MST Test Services
Establish Alternative Criteria
No
Meeting Criteria?
Yes
Resolution
Three-legged stool

Thank you!

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Source Molecular

ilac-MRA ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory