



# 09-NGI-12 - Pathogen Detection and Canine Fecal Source Identification: Validation of Assays to Identify Areas Requiring Management Action

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Detection of Noroviruses



Canine Fecal Source ID

# BEACH Act 2000

- Develop new indicators of fecal pollution and methods to monitor beach water quality
- Assess human health risk
- Provide guidance to beach managers
- Beach Protection Act 2008 - improve and expand beach monitoring & identify pollution sources



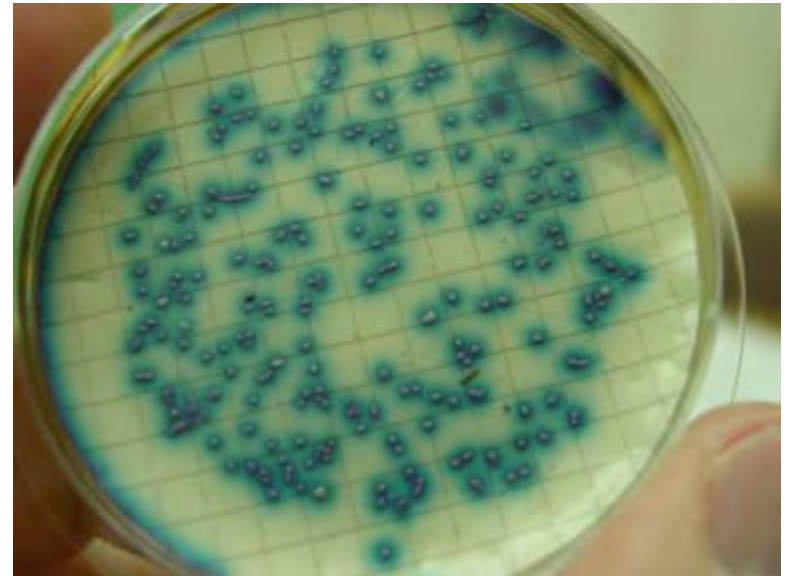
# Beach Monitoring

- EPA sets the guidelines for testing but local governments do the testing
  - Mississippi - Dept of Environmental Quality
  - Alabama – Dept of Environmental Management
  - Louisiana – Dept of Health and Hospitals
- Funding for testing via annual fed grant

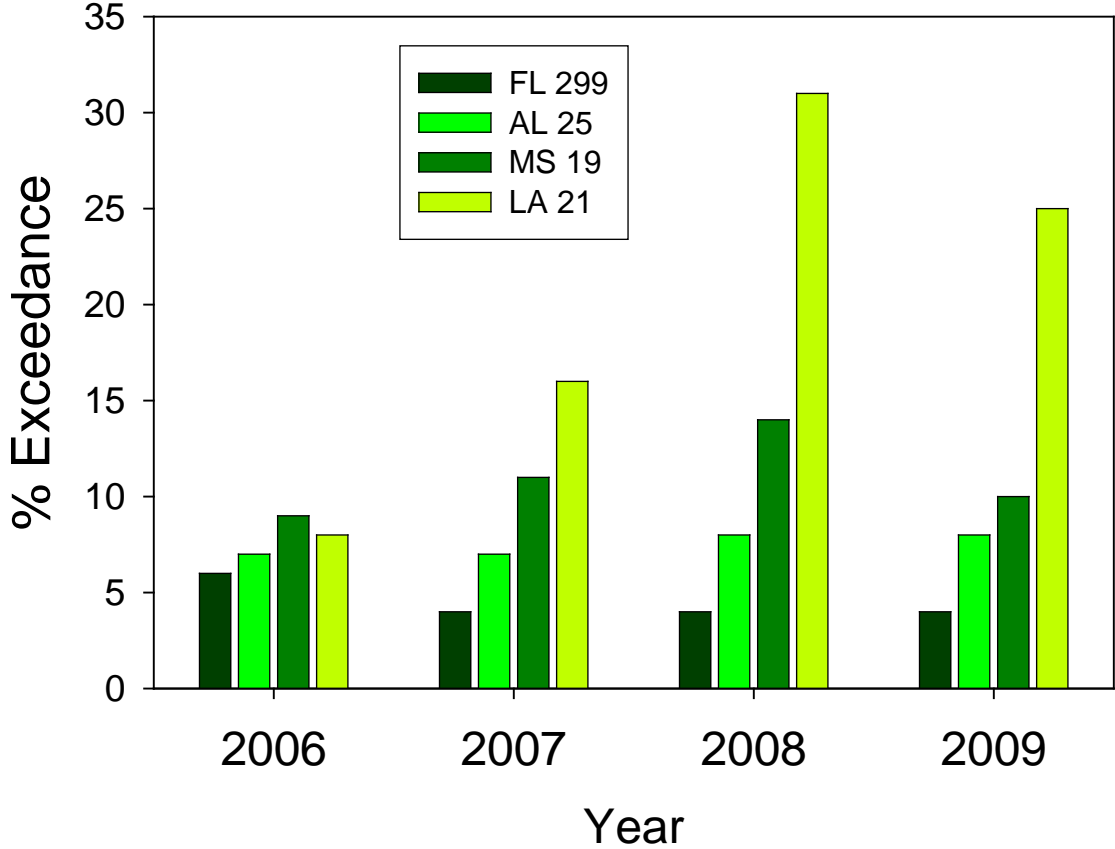


# Current Method

- Plate counts using fecal surrogate - *Enterococcus*
- Single-sample maximum of 104 cfu/100ml
- Geometric mean of 35 cfu/100ml over 5 samples/days



# How are the beaches doing?



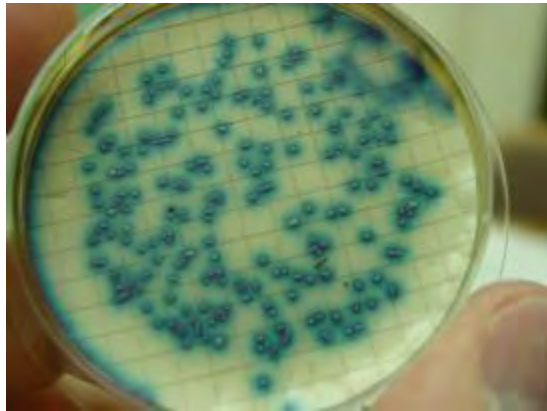


- Source of pollution almost never identified
  - Rain - wildlife and agricultural runoff
  - Faulty septic systems
  - Confined animal feeding operations
  - Sewer overflows



# Current method

- Plate counts using fecal surrogate - *Enterococcus*



## Problems

- No indication of fecal source
- Retrospective
- Persistence

## Why it is still being used

- Reliable assay to perform
- No new EPA standard



# Source Identification Validation Study

- Many candidate methods but few evaluation studies
- California is looking to standardize source identification methods
  - Assembly Bill 538 requires the State Water Resources Control Board to develop a guidance manual





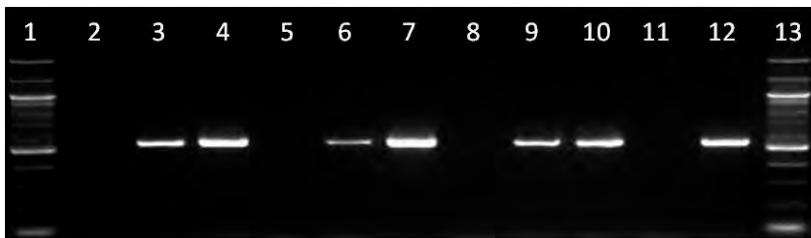
# Promising & Exploratory PCR-based Methods

- Human Markers

- Bacteroidetes
  - HF 183
  - Hum M3
  - *B. thetaiotamicron*
  - *B. fragilis* gyraseB
- *Methanobrevibacter smithii*
- Adenovirus
- Enterovirus
- Norovirus
- Bacteroidetes phage
- Human polyoma virus
- Somatic coliphage

- Animal Markers

- Bacteroidetes
  - Cf 128 & 193
  - DogBac (dog)
  - Cow M3
  - *Rum-2-Bac* (cow)
  - HoF597 (horse)
- *Methanobrevibacter ruminantium*
- *Catellibacoccus marimammalian* (gull)
- Bovine & porcine adenovirus
- Porcine methanogen *mcrA*
- Bovine polyomavirus
- Pig and sheep mitochondrial markers



Developed with NGI support

# Approach

- Challenge methods with 64 blind samples
  - Singletons and doubletons of 12 source types
- Core methods run by more than one lab
  - Want to understand method repeatability
- 36 methods to be evaluated
  - 29 participating laboratories



# Participating Labs

- Ali Boehm, Stanford
- Jenny Jay, UCLA
- John Griffith, SCCWRP
- Trish Holden, UCSB
- Stefan Wuertz, UC Davis
- Jed Fuhrman, U Southern California
- **Chris Sinigaliano, U Miami**
- Rachel Noble, U North Carolina
- Mike Sadowsky, U Minnesota
- Jill Stewart, U North Carolina
- Gary Anderson, UC Berkeley
- Jiyoung Lee, Ohio State U
- Joan Rose, Mich State U
- Vijay Kannappan, Wayne State U Michigan
- **Jody Harwood, U South Florida**
- Huw Taylor, U of Brighton, UK
- Wim Meijer, U of Dublin, Ireland
- Andreas Farnleitner, Vienna U of Technology, Austria
- Melanie Wicki, Federal Office of Health, Switzerland
- Michele Gourmelon, Ifremer Laboratoire de Microbiologie Plouzane France
- Raquel Rodriguez, National Institute of Health, Portugal
- Orin Shanks, EPA
- **Kelly Goodwin, NOAA**
- Jorge Santo Domingo, EPA
- Murulee Byappanahalli, USGS
- Theng Fong, Tetra Tech
- Maurice Larenas, Source Molecular
- Scott Reynolds, Environmental Canine Services

# Progress

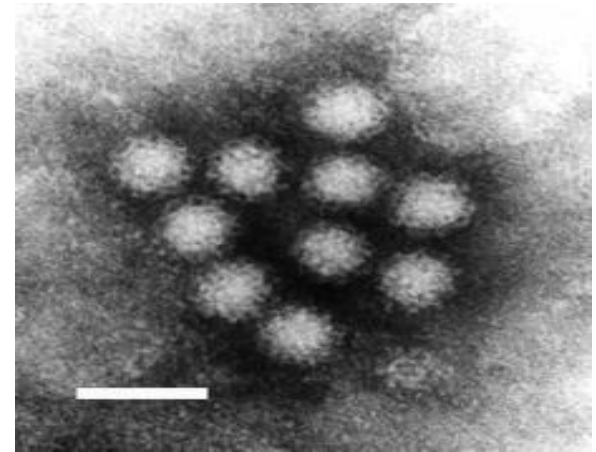
- Sample preparation - Jan 25
- Results due to us - March 1
- Meeting of participants to discuss results - May 21

Stay Tuned to See Which  
Methods Transition To  
Operations!



# Noroviruses in Coastal Waters

- Monitor pathogens instead of surrogate
  - NoV – cause of 21 million cases of acute gastroenteritis each year
    - 57% foodborne
    - 16% person-to-person spread
    - 3% waterborne
    - 23% not determined



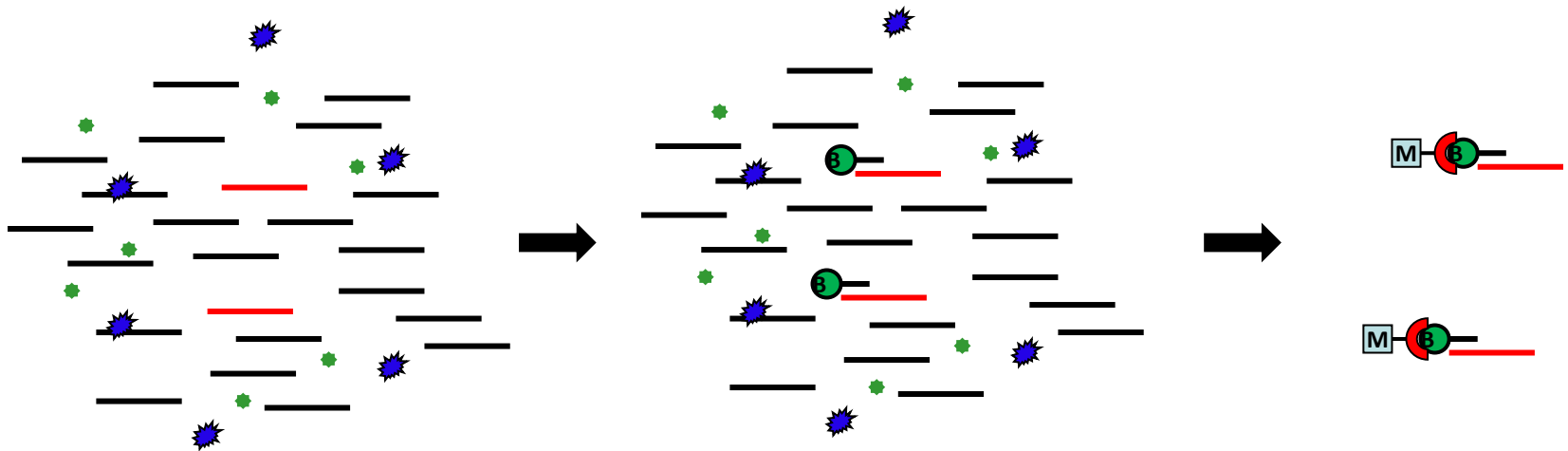
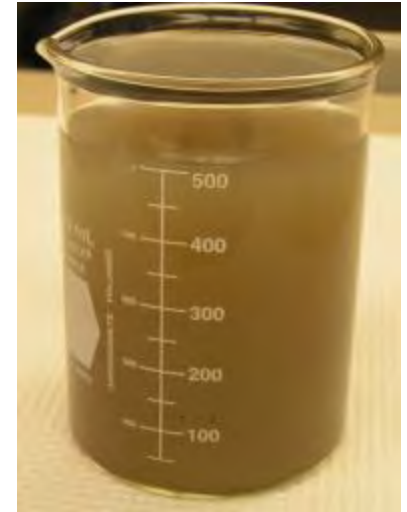
# Detecting Noroviruses in Water

- Importance
  - High infectivity
  - Environmental stable
  - Cause sudden outbreaks
  - No treatment or vaccine
- Challenges
  - Extremely dilute
  - Genetic diversity



# Progress

- Developed a RNA capture method to improve sensitivity of RT-PCR assay



# Progress

- Developing the use of *Corbicula fluminea* as a sentinel of NoV contamination in coastal creeks





# Acknowledgements

- Xunyan Ye
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- EPA Gulf of Mexico Program
- NGI
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