

# Fecal Indicator Bacteria Methods: The Good, Bad and Ugly

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# Learning Objective

To better understand the significance of fecal indicator bacteria (FIB) in environmental waters given their limitations as indicators of fecal pollution, shortcomings of FIB test methods, biological variability and ecology of FIB.

# Overview

- Fecal Indicator Bacteria (FIB)
  - Assessing environmental waters (beaches, creeks, storm water)
  - Utility and limitations of *indicators*
- Commonly Used FIB Testing Methods
  - Utility and limitations of *methods*
- Proposed Regulations
- Advanced Indicator Methods
- Summary

# Background

- Fecal Indicator Bacteria (FIB): groups of bacteria normally present in the intestinal tracts of humans, animals & birds
  - Total Coliforms (TC), Fecal Coliforms (FC), E. coli (Ec) & Enterococci (Ent)
- FIB are used to assess
  - Sanitary quality of water
  - Potential human health risks
  - Fecal sources of contamination
  - Effectiveness of risk reduction actions
- TC were the first group of indicators of used for testing, followed by FC, Ec and Ent

# CA Beach Water Quality Criteria or Limits (counts per 100 ml water)

- Single sample
  - TC 10,000
  - FC 400
  - Ent 104
  - TC to FC ratio  $<10$
- Geomean (at least 5 weekly samples during any 30-day sampling period)
  - TC 1,000
  - TC 200
  - Ent 35

# Total Coliforms

- Are the largest group of FIB
- Includes FC and Ec
- Rationale for using TC: TC are present in higher numbers in the guts of humans and other warm-blooded animals as compared to FC and Ec, if water is contaminated by fecal waste, TC can be detected even after dilution; FC and Ec may not be detected
- Limitations: TC are not specific to fecal waste; many TC exist and proliferate in soil and water, as well as in treated drinking water systems

# Fecal Coliforms

- FC was recommended as being more specific to human fecal waste compared to TC
  - represent a smaller group of bacteria as compared to than TC
  - can grow at a higher temperature (similar to human body temperature)
- Limitations: FC are not specific to fecal waste; many FC exist and proliferate in soil and water, drinking water systems

# E. coli

- E. coli was recommended as being more specific than FC because it represents one species
- *E. coli* is a predominant group within the FC coliform group
- Limitations: E. coli is not strictly limited to warm-blooded hosts; has been observed in the feces of reptiles; recent studies show Ec can grow on plant surfaces, beach sand and beach wrack

# Enterococci

- Suggested to be better indicator than TC, FC and Ec because enterococci have similar (or greater) survival rates in water as some pathogens
- Limitations: Ent are not specific to fecal waste; they are ubiquitous in the environment, cultivatable from soil, sand, plant surfaces, and insects; found in food and other human body sites (oral cavity, vagina and occasionally, urinary tract)

# The Good

- FIB have been reliable for monitoring
  - drinking water
  - sewage spills in receiving waters
  - comparing FIB levels upstream and downstream in streams receiving sewage effluent

# The Not So Good, Bad & Ugly

- Have FIB been reliable for monitoring environmental waters?

# Do TC, FC, Ec and Ent Meet Bonde's (1966) Criteria for an Ideal Indicator?

<b>BONDE'S CRITERIA FOR IDEAL INDICATORS</b>	<b>Answer (in general)</b>
Should always be present in feces of humans and warm-blooded animals	<b>Yes</b>
Must not be able to multiply in aquatic environments	<b>No</b>
Occur in much greater numbers than the pathogens	<b>Yes and No</b>
Must be unambiguously identifiable by simple, characteristic and reliable tests	<b>Yes and No</b>

# Reoccurring Problem

- New and “better” FIB were introduced *before* researching their ecology (i.e. range of sources, habitats, survival, growth) in the environment
- Molecular markers have been introduced as an alternative to FIB; however, few studies address their ecology

# FIB Methods: Membrane Filtration (MF) and IDEXX

- Widely used
- Now considered “traditional” methods
- Culture based, not molecular

# Desirable Attributes of Indicator Methods

- Specific to desired target organism
  - Independent of matrix effects
- Applicable to different water types (salinity, turbidity)
- Precise/reproducible
- Adequate sensitivity
- Rapid
- Quantifiable
- Easy to perform
- Low cost

# IDEXX

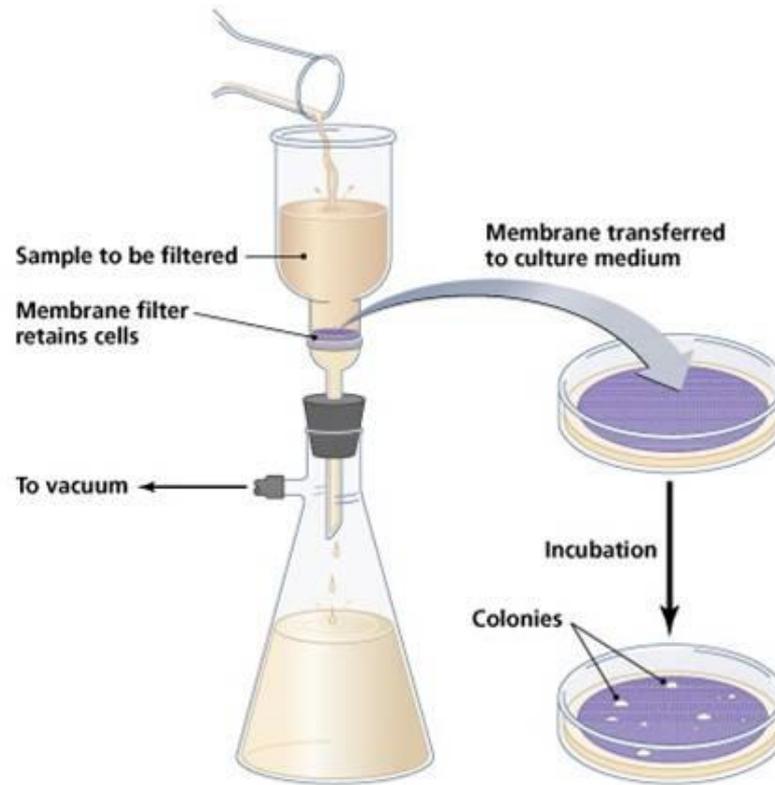
- Methods: Colilert, Colilert 18, Enterolert
- IDEXX is simple and less time consuming to perform as compared to membrane filtration, thus preferable for high volume testing
- Detects indicators in 24 hours or less



# IDEXX

- Accurate & no confirmation needed\*
  - \*Confirmation may be needed at sites that repeated exceed standards; no protocol from IDEXX—search scientific literature
- Quanti-Tray 2000 allows counts up to 2,419 MPN (no dilution)
- Cost for equipment, culture media and labor are higher for MF
- Interpretation of results (fluorescence) can be more subjective than MF, leading to false positives

# Membrane Filtration (MF)



(a)

# Membrane Filtration (MF)

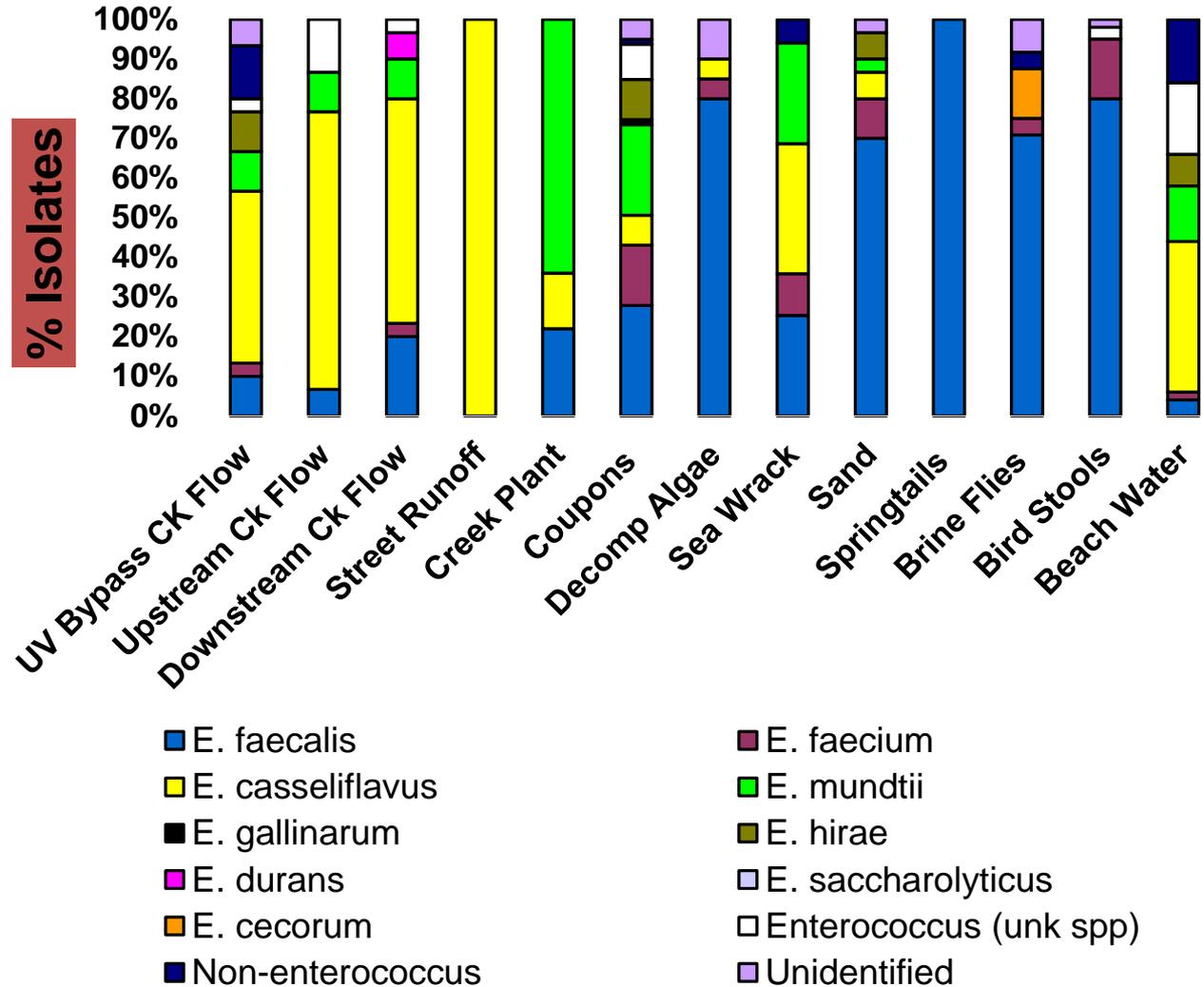
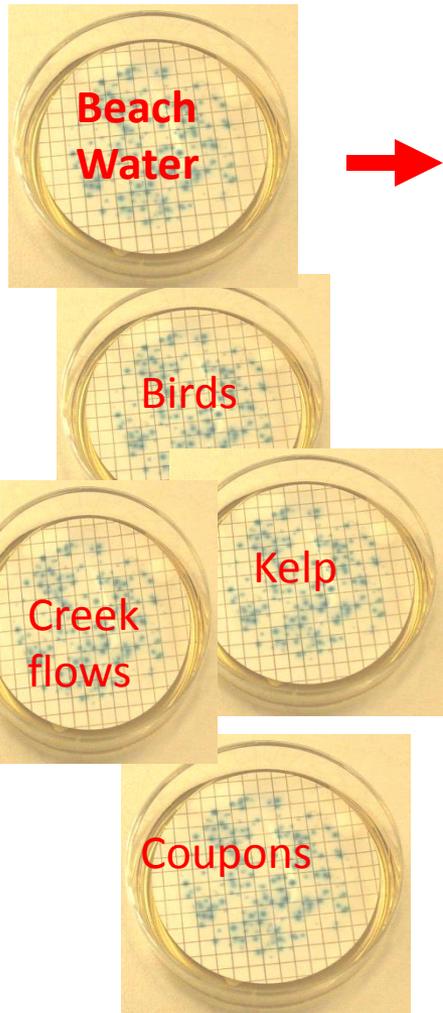
- Methods (media)
  - TC (mENDO)
  - FC (mFC)
  - E. coli (mTEC)
  - Ent (mEI)



# Membrane Filtration (MF)

- Requires higher level of expertise
- More time consuming than IDEXX
- Higher cost for equipment & media
- Turbid samples can clog filter and mask bacteria
- MF allows further characterization of FIB testing, which may be useful for assessing potential source contributions
  - Species identification
  - Strain (sub-species) typing

# Speciating Isolates Obtained Using MF



# Method Limitations

- FIB counts may be under- or over-estimated using MF and IDEXX
  - Results may vary depending on
    - water type and sample location
    - method
    - volume of water tested
- IDEXX Colilert
  - City of Santa Cruz reported IDEXX Colilert overestimated TC counts for 6 beaches
  - Other studies have also reported false positive results using Colilert for marine water

# Method Limitations (cont)

- The volume of water tested matters. In most cases, the greater the volume tested, the more representative the count
- FIB counts are reported per 100 ml of water; thus, testing low volumes can lead to overestimating counts due to extrapolation errors
- Filtering high volumes of turbid water samples can lead to underestimating counts due to filter clogging or masking of bacteria

# Method Limitations (cont)

- Insufficient shaking of samples prior to testing may lead to inaccurate counts.
  - Bacteria are not homogeneously distributed in water samples and may be attached to other bacteria and particulates
  - 100 ml of water sample may yield 15 total coliforms, but 10 ml (1:10 dilution) of the same sample may yield also yield 15 total coliforms
- Both methods detect bacteria that are not considered TC, FC, Ent (false positives)

# Why do FIB Counts using IDEXX and MF Differ?

- Bacteria grow differently in broth (liquid) and agar
  - MF: agar
  - IDEXX: broth
- The units of measurement differ between IDEXX and MF
  - MF: colony forming units/ml → actual count
  - IDEXX: most probably number/ml → statistical count

# Why do FIB Counts using IDEXX and MF Differ (cont)?

- Not all FIB methods measure the same population (genus and species) of organisms or same proportion of genus and species
- Inter-lab and inter-technician variability
- Biological variability
  - Counts from duplicate samples vary
  - Samples taken 10 minutes apart may differ

# Why are we still using FIB?

- Methods are cheap and easy to perform
- Some epidemiologic studies have shown a correlation between FIB counts and illness rates
  - Limitations: Even epidemiology studies have had shortcomings
    - Results of earlier epidemiologic studies were based on FIB methods that were less specific than currently used methods. Also, the same methods were not used across all studies.

# Inconsistencies Between Epi Studies

- A review of recent epi studies found that enterococci had the best relationship to health risk among presently used indicators for marine water, but less than half of the studies found a significant health relationship and the dose-response curves establishing the relationship between increased illness and indicator density were highly variable.
- National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) Epidemiology Studies showing good correlation between Ent and illness rates were conducted at sites impacted by point sources with known human fecal contamination, which may not represent beaches elsewhere.
- Recent epidemiological studies at beaches in California impacted primarily by urban runoff and/or natural sources did not find a correlation between FIB levels and illness rates

# Proposed Ocean Monitoring Regulations

- For water quality assessment and TMDLs (not for AB411 monitoring)
- Proposal
  - Enterococci and E. coli alone rather than total and fecal coliforms
  - Enterococci for marine waters and E. coli for freshwater

# Potential Limitations to Using a Single Indicator

## Enterococci

- Widespread occurrence and ability to persist in the environment may confound assessment of fecal contamination and remediation efforts
- Comparing Ent counts with Ec and/or FC can shed more insight as to sources
  - Elevated Ent levels can occur in the absence of fecal contamination due to natural sources such as plants, soil, etc. and growth in the environment. If Ent counts are elevated but Ec or FC levels are non-detected or low, this *suggests* natural source. If Ec and FC are also elevated, this may be *more indicative* of fecal contamination

# Multiple Indicators Can Provide More Information

- Observations based on years of FIB monitoring southern CA beach and storm water:
  - After sewage spills, counts for TC, FC and ENT levels are ALL high
  - At sites impacted by high bird densities, TC and FC counts are generally higher than Ent
  - In storm water or from water bodies near aquatic vegetation, it's not unusual to find Ent counts higher than FC and Ec. Consistently high Ent counts suggest plants or soil may be a primary contributors; however, fecal waste from animals that consume plants must also be considered

# Considerations for assessing swimmability at beaches given these limitations

## Know your beach

- What are the potential sources of fecal contamination?
- What are the typical background levels of FIB?
  - Are Ent levels consistently near or above water quality limit (104 ss, 35 geomean)? Is there a constant source of Ent?
  - Are increases in FIB counts due only to random fluctuation or biological variation?
- Could the variability in FIB counts reflect changes in testing methods, testing lab, testing personnel?
- Is using IDEXX appropriate at your beach?
  - What organisms is your test detecting?
  - What is your false positive rate?
  - What proportion of FIB counts reflect non-FIB or environmental strains?

# Enterococcus qPCR

- Overview
  - Enterococcus qPCR as an Approved Method
  - Overview of qPCR
  - Advantages and Disadvantages
  - Implementation of Enterococcus qPCR
  - California labs that have qPCR capability
  - Practical uses for molecular methods in our lab



# Overview of qPCR

## Rapid Microbiological Monitoring Methods

A Fact Sheet from the Southern California Coastal Water Research Project



January 2011

*Rapid methods could improve the accuracy and speed of beach warnings.*

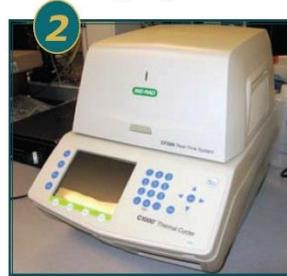
### Why use rapid methods?

Tests are complete in 2-4 hours, allowing same-day posting of beach water quality information. qPCR, the most advanced rapid method to date, shortens testing time by detecting and quantifying DNA from the microorganisms of interest, rather than waiting for them to grow.

### How does it work?

qPCR = quantitative polymerase chain reaction

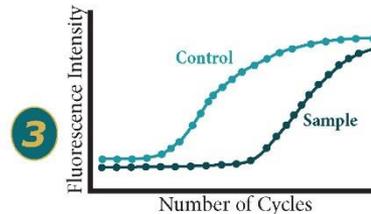
DNA replicates itself as part of many normal biological processes, and qPCR imitates and speeds up this process in the laboratory.



**Step 1:** Bacteria in the water sample are captured on a filter and mixed with reagents that simulate normal DNA replication.

**Step 2:** Fluorescent probes that adhere to the new DNA copies are added, and the sample is subjected to repeated heating and cooling cycles to double the amount of DNA every few minutes.

**Step 3:** A computer records the amount of fluorescence in real time to estimate how much DNA, and how many cells, were present in the original sample.



# Enterococcus qPCR

- Advantages
  - Rapid – potential for same day results
  - Specific – assays can be designed to target very specific organisms with minimal to no cross reactivity
  - Correlation with culture method
- Disadvantages
  - Cost
  - Complexity
  - Standardization
  - False positives due to DNA contamination
  - Results may not be available until afternoon, precluding same day warning to swimmers
  - Lower throughput of samples tested per day compared to IDEXX and MF

# Correlation with culture methods

- Most of the comparison studies done in the last 10 years showed good correlation between qPCR and culture methods when you have positive samples
- Reasons for discrepancies
  - Measuring different endpoints
    - Culture methods – detect viable organisms for growth
    - qPCR – detects amplification of DNA from viable and nonviable organisms
  - Calculation method
    - dCT vs ddCT
  - Inhibition

# Cost

- Equipment cost (\$65,000 - \$135,000)
  - qPCR Instrument \$35,000-\$100,000
  - Centrifuge (micro & mini) \$6,000-\$7,000
  - Bead beater \$15000
  - Vortex \$1,200
  - Pipettes \$6,000-\$7,000
  - PCR workstation \$2500
- OCPHL Cost per sample
  - One target \$85.12
  - each additional target \$37.38

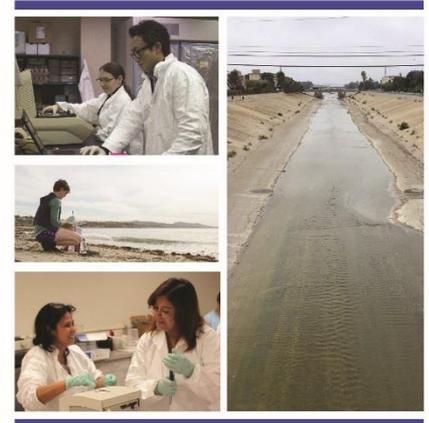


# Complexity

- Technology transfer
  - BIGHT '13 – SCCWRP trained 14 water agencies to perform Enterococcus qPCR and HF183 qPCR
    - Short-term - Labs maintained proficiency through the intercalibration study
    - Long-term proficiency was not maintained
- Need to perform regularly to maintain proficiency



## Shoreline Microbiology



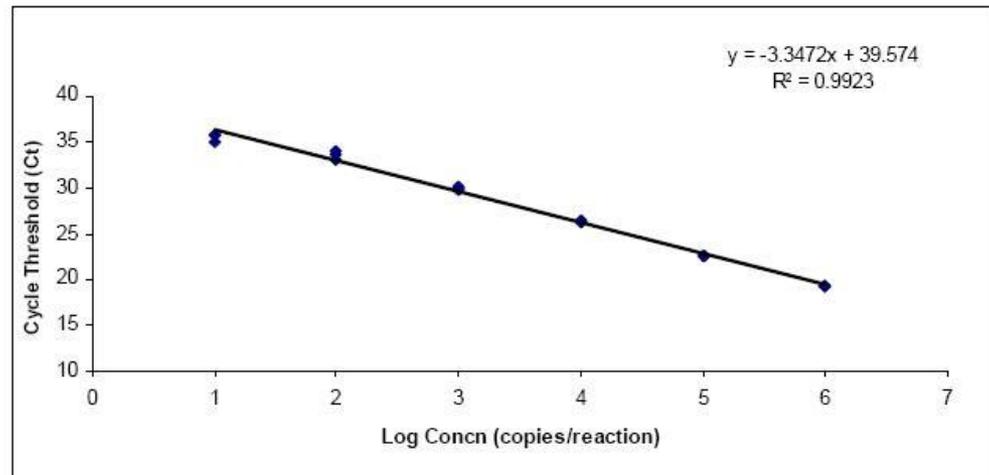
BIGHT '13

Southern California Bight  
2013 Regional Monitoring  
Program  
Volume IX

SCCWRP Technical Report XXX

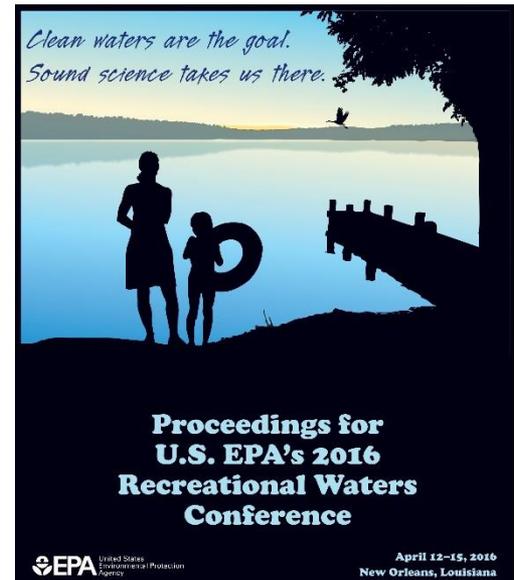
# Standardization

- Standard curve
  - EPA method provides procedure for labs to make their own DNA standards
  - DNA standard concentration varies from lab to lab
  - Difficult to replicate the standard curve from one lab to another even when using the same DNA standards



# qPCR Implementation

- 2016 EPA Recreational Water Conference
  - Michigan
    - Statewide implementation of E. coli qPCR at 16 labs
  - Chicago
    - Compared Enterococcus qPCR to E. coli culture at 5 beaches
  - Hawaii
    - Enterococcus qPCR at 12 beaches
    - 69% of samples were inhibited



# CA Labs with Enterococcus qPCR capability

- 8 labs in So. Cal. trained to do qPCR
  - Southern California Coastal Water Research Project
  - Ventura County Public Health Laboratory
  - Los Angeles County Sanitation District
  - City of Los Angeles Environmental Monitoring Division
  - Orange County Sanitation District
  - Orange County Public Health Laboratory
  - Weston Solutions
  - City of San Diego Public Utilities Department
- CDPH – Drinking Water & Radiation Lab in Richmond, CA
- Humboldt County PHL – shellfish testing qPCR for Vibrio

# Practical uses for molecular methods at OCPHL

- Enterococcus qPCR
  - Routine beach monitoring
    - Select sites with higher rate of exceedances
    - If using rapid methods it's recommended to test the site more than once a week
  - Post exceedance re-check samples to reduce turnaround time to un-post beaches
  - Sewage spill samples to reduce beach closure days
- Source tracking

# Future Considerations

- Advances in science and technology will continue to result in the introduction of more complex, expensive, and difficult-to-interpret tests
- New methods advance quickly; need time for rigorous review and improvements for implementing for regulatory use
- New methods should be used as “RUO” until proven and widely used
- Widespread use of new methods often reveal limitations
  - Ex. Alm et al., 2017 showed HF183 human marker is present in bird stools. This confirms study conducted in Orange County at Doheny beach
- Be patient but vigilant
  - Pressure on regulatory agencies to approve advanced methods quickly may hinder rigorous validation

# Summary

- Understanding the limitations of FIB and testing methods is critical to making more accurate assessments regarding fecal contamination
- Avoid FIB testing abuse
  - Focusing on FIB counts or results based on a single indicator to assess water quality may be misleading
  - Verify questionable results
  - Identify the organisms being measured
- Always correlate FIB data with source inputs and environmental conditions and also consider biological variation
- Enterococcus qPCR can yield results in a few hours; useful for re-opening posted beaches quickly; results and data interpretation may vary between labs and beach sites

# Questions?

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# References

- INDICATORS FOR WATERBORNE PATHOGENS <https://www.nap.edu/read/11010/chapter/6>
- Jang, et al., 2017. Environmental Escherichia coli: ecology and public health implications—a review. J. Appl. Microbiol. doi:10.1111/jam. 13468
- <http://www.sccwrp.org/ResearchAreas/BeachWaterQuality/CaliforniaEpidemiologicalStudies.aspx>
- Pisciotta, et al, 2001. Marine bacteria cause false-positive results in the Colilert-18 rapid identification test for Escherichia coli in Florida waters. AEM
- Landre, et al., 1998. False-positive coliform reaction mediated by Aeromonas in the Colilert defined substrate technology system: J Appl Micro
- <http://www.sccwrp.org/ResearchAreas/BeachWaterQuality/ComparisonAmongIDEXXMembraneFiltration.aspx>
- <http://www.idexx.com.au/water/products/ quanti-tray.html>
- Stoeckel, D. 2005. Selection and Application of Microbial Source Tracking Tools For Water-Quality Investigations, USGS. [https://pubs.usgs.gov/tm/2005/tm2a3/pdf/Book2\\_Collection%20of%20Environmental%20Data.pdf](https://pubs.usgs.gov/tm/2005/tm2a3/pdf/Book2_Collection%20of%20Environmental%20Data.pdf)
- Ferguson et al., 2013. Comparison of Enterococcus species diversity in marine water and wastewater using Enterolert and EPA Method 1600. J Env & PH <https://www.hindawi.com/journals/jeph/2013/848049/>
- Ferguson et al., 2016. Enterococcus growth on eelgrass (Zostera marina); implications for water quality. FEMS 92