# Discussion Topic 2: Rapid Methods

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## Ambient Water Quality Criteria

- 1986 Criteria: EPA Method 1600 for enterococci and EPA Method 1603 *E. coli.* Other vendor methods have been approved (e.g., IDEXX methods)
- 2012 Criteria: Rapid (qPCR) for beach notification and monitoring; and rapid and/or culture methods for compliance with other CWA programs.



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## Stakeholder Input

- Criteria should be based on indicator/methods that are correlated to public health impacts.
- Criteria should work across multiple water programs (NPDES, TMDL).
- For beach notification, indicator/method should provide timely results.
- EPA should strive to develop new methods that cost the same or less than current methods.
- EPA should provide options for use of alternative indicators and analytical methods.

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## qPCR Basics

- What is qPCR?
  - Quantitative Polymerase Chain Reaction.
- What are PCR and qPCR tests used for?
  - Genetic tests used to identify (PCR) and quantify (qPCR) DNA or RNA strands from microorganisms, plants, animals.
- How does qPCR work?
  - It chemically replicates the DNA strands, generating a fluorescent signal that can be detected. The signal is quantifiable, hence qPCR.
- Advantages
  - Faster - hours versus day(s).
  - Easier to identify specific strains.
- Disadvantages
  - Doesn't differentiate live versus dead cells.
  - More challenging technically than culture methods.

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# Method Comparison (1)

qPCR

1. Collect water sample.
2. Filter water sample through membrane filter (different kind from culture samples).
3. Extract DNA from the filter.
4. Add chemicals to tube containing extracted DNA.
5. Place tube in qPCR machine and run machine.
6. Import data from machine into spreadsheet for calculations.

Culture

1. Collect water sample.
2. Filter water sample through membrane filter.
3. Place membrane in selective medium.
4. Incubate for 24 hours.
5. Count and record colonies => 0.5mm diameter.



# Method Comparison (2)

- For culture methods, 3 types of cells on the plate:
  1. viable cells: live cells
  2. injured cells: cell wall intact but cell will not grow
  3. dead cells: cell intact but not viable
- Most culture methods will only pick up subset of cells in #1 and with resuscitation step, cells in #2.
  - For example, *E. coli* culture test includes "resuscitation" step to move injured cells into the viable cell category.
- qPCR will pick up all 3 types of cells.
  - More sensitive to detect cells that may cause illness.
  - May not be a good way to measure the efficacy of disinfection.

# EPA's Current Thinking

- EPA may recommend a qPCR method which would be used for beach advisories and notification purposes and qPCR and/or culture for other CWA applications.
  - Use of molecular methods may not be necessary permitting and implementation of TMDLs.
- Ideally, EPA would like to use the same indicator organism for both qPCR and culture methods.
  - This could be done by using epi study results or by "linking" any indicator/method combination and its corresponding concentration to a single health risk level.
    - Example: Ind.A/culture = 50cfu/100ml at Y/1,000 illness and Ind.B/qPCR = 10CCE/100ml at Y/1,000 illness
    - Paired indicator/methods will be easier to explain to the public.

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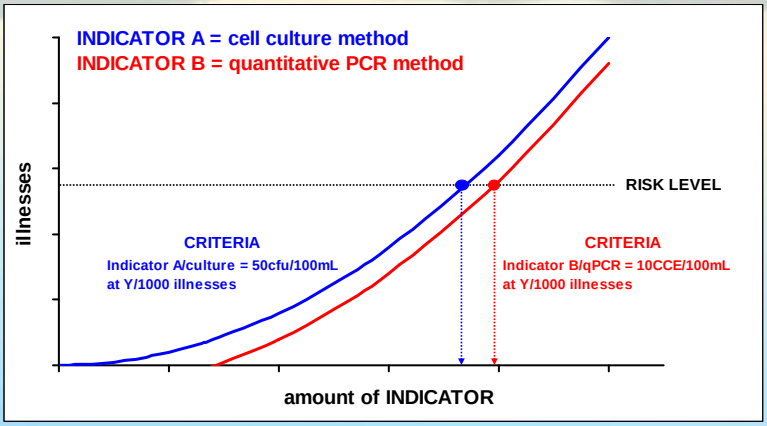
# Example: Linking Indicator/Methods Using Single Health Risk Level

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# Method Characteristics

- Characteristics of indicator/methods which are good candidates for new recreational water quality criteria:
  - Organism is a good indicator of fecal contamination.
  - Strong correlation to illness in recreational waters.
  - Specificity, maturity of the method (what is known about method performance) and method complexity.
  - Rapidity of the method.

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# Promising Indicator/Methods

Methods that have demonstrated a relationship to GI illness based on results to date.

| Indicator method | 1986 | EPA Great Lakes* | EPA marine | EpiBathe** |
|---|---|---|---|---|
| Enterococcus | | | | |
| Culture | yes | no | | yes |
| qPCR | n/a | yes | | n/a |
| *E.coli* | | | | |
| Culture | yes[1] | n/a | | yes[3] |
| qPCR | n/a | n/a | | n/a |
| Bacteroidies | | | | |
| qPCR | n/a | no[2] | | n/a |
| Clostridium | | | | |
| Culture | n/a | n/a | | n/a |
| Coliphage | | | | |
| Anti-body assay | n/a | n/a | | n/a |

***Does not include results of archived frozen filter analysis for qPCR methods**

**** Information can be found at: : www.epibathe.eu**

[1] Relationship in freshwater but not marine.

[2] No association in Wade et al., 2006, insufficient data to infer association in Wade et al., 2008

[3] The Epibathe study found no association with *E. coli* and GI illness in marine waters and a loose association in freshwaters.

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# Indicator / Methods Under Testing

| EPA Study | Indicator/Methods Tested in Study |
|---|---|
| Great Lakes epi | Enterococcus-qPCR, Enterococcus-culture, Bacteroides-qPCR |
| 2007 Marine epi | Enterococcus-qPCR, Enterococcus-culture, E. coli-qPCR, Bacteroides thetaiotamicron (human specific)-qPCR, Bacteroides (general/non-human specific)-qPCR, Male-specific Coliphage by antibody assay, Clostridium spp.-qPCR |
| Archived filters* | Enterococcus-qPCR, E. coli-qPCR, Bacteroides (general/non-human specific)-qPCR, Clostridium spp.-qPCR, human associated markers |
| Tropical epi | Same as 2007 marine, but no Coliphage |
| Urban Runoff epi | Same as 2007 marine, but no Coliphage |

*Analysis of culture methods will not be performed on the archived frozen filters for the epi studies since holding time has been exceeded.



# Remaining Analysis (1)

- Results from EPA epi studies:
  - 2007 Marine studies
  - Tropical
  - Urban Runoff
- Results from non-EPA studies:
  - SCCWRP studies
  - University of Miami study
  - European Studies/Epibathe
  - Others

## Remaining Analysis (2)

- EPA is conducting work to determine if and how results from studies which use different designs can be compared, combined and synthesized.
  - Epidemiological studies use 2 basic study designs:
    - **A prospective cohort study** (PC) which follows over time a group of individuals who are alike in many ways but differ by a certain characteristic (for example exposure to recreational water) and compares them for a particular outcome.
    - **Randomized controlled trial** (RCT) is a study in which people are allocated at random (by chance alone) to be exposed to recreational waters.
- This will allow us to consider results from a wider range of epi studies in the development of new criteria.

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## Remaining Analysis (3)

- Conduct single and multi lab validation studies for promising methods.
  - Single lab study underway currently for enterococcus qPCR and bacteroides qPCR.
  - Multi lab validation study planned for next year.
- Complete analysis of archived frozen filters from past epi studies.

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## Remaining Analysis (4)

- Identify, collect and collate studies in literature and state/local sponsored reports on methodologies to enumerate fecal indicators.
  - Special focus on studies/reports where both culture and molecular methods have been used.
  - Develop a matrix table to characterize pertinent types of data (e.g., waterbody type, nature of fecal sources, fecal indicators and methods used).
- Conduct analysis to compare datasets.
  - Statistical assessment of acceptability of correlation for comparison of fecal indicator-to-fecal indicator and for fecal indicator-to-health risk correlation, such as:
    - Correlations of different indicator/methods for different water body types.
    - Comparison of rates of false (+) and false (-) results.
    - Relationship of indicators/methods within and across studies to each other and to health risks.



## Opportunities & Challenges (1)

- Pending BEACH Act legislation includes new provisions:
  - Requirements related to publication and use of "rapid testing method" for beach advisories/notification programs by states with EPA grants.
  - EPA promulgation of new or revised water quality standards if states do not adopt EPA recommendations within 3 years (i.e., by October 2015).
    - Federal promulgation of water quality standards could limit state's ability to fully utilize any flexibilities which may be described in EPA's recommendations.

## Opportunities & Challenges (2)

- EPA will need to synthesize data from a wide assortment of studies which may have conflicting or inconsistent results regarding indicator/method relationship to illness.
- Culture-based methods require 24-48 hours to obtain results. qPCR is a faster method to assess recreational water quality and predict swimming-related illnesses,
  - Even with rapid testing method, beach notification decisions could not be made for 4-6 hours after sample collected.
- EPA is considering the use of predictive models to supplement beach monitoring, but not to replace it.
- EPA is considering developing a process/methodology for incorporation of new methods into future criteria development in the absence of an epi study.

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## Questions for Discussion Respondents & Audience

- What are the opportunities and challenges in using rapid methods for beach programs while using rapid and/or culture methods for compliance with permitting, listing decisions and TMDL development?
- How do you envision the use of historical data in transitioning to a new criteria?
- What tools and training would be needed to ease implementation of molecular methods?

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