

Implementation of ddPCR for Beach Water Quality Monitoring in San Diego County

Presentation to The SCCWRP Commission

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Background

- Growth-based methods for monitoring beach water quality are too slow
 - SCCWRP has been leading the charge to develop, evaluate and implement rapid methods for same-day water quality results for over 20 years
- PCR-based methods have shown the most promise
 - US EPA developed and published a rapid qPCR method for *Enterococcus* in 2012
 - SCCWRP adapted EPA's qPCR method to digital PCR in 2014
- PCR-based methods for beach monitoring are in use in the Great Lakes and Florida
 - San Diego County Health is the first in the nation to use droplet digital PCR measurements of *Enterococcus* for daily monitoring

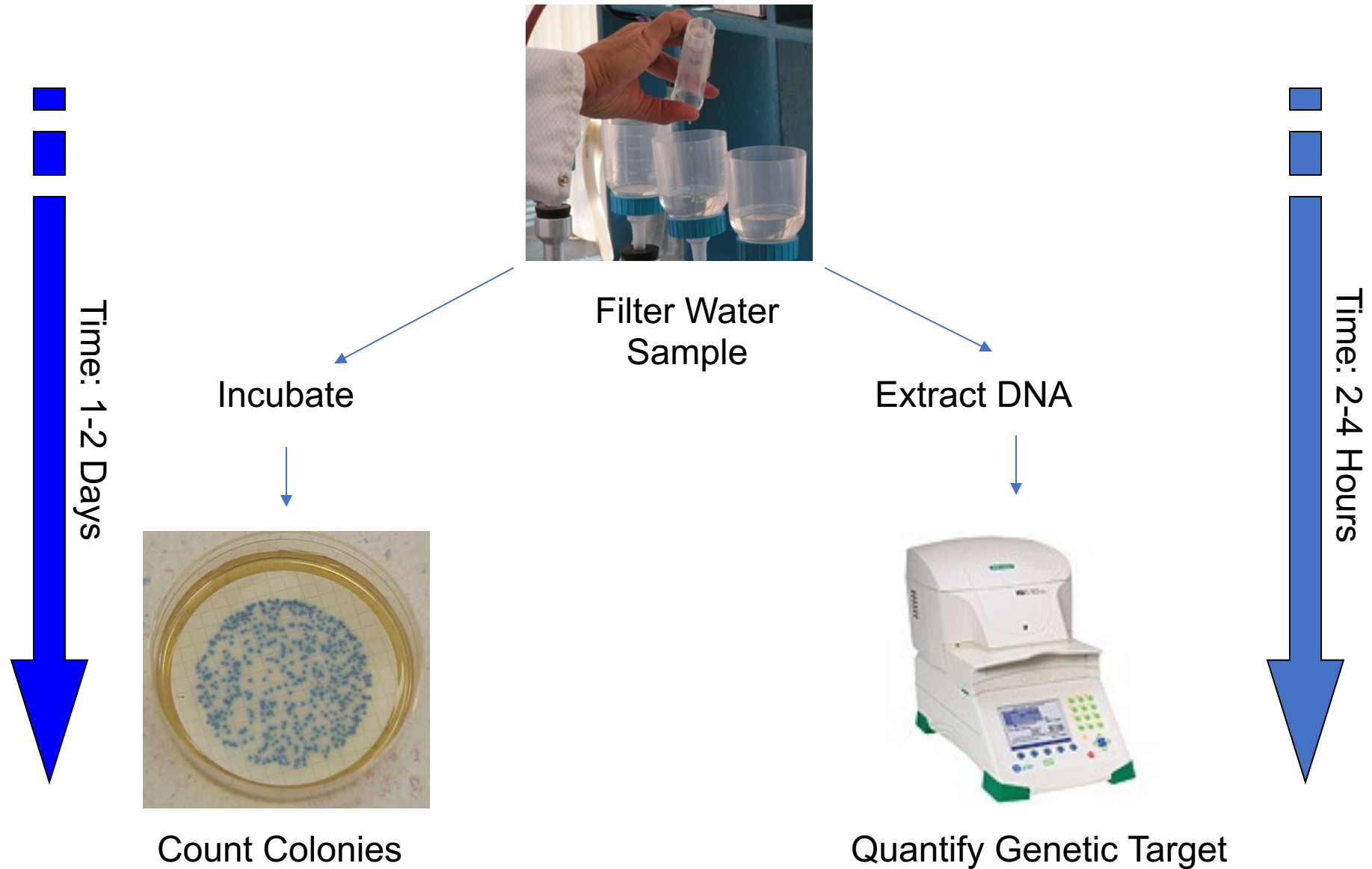
Timeline

- 1999 – Beach Act requires US EPA to develop and publish rapid method for measuring fecal indicator bacteria in beach water
- Early 2000's – SCCWRP holds Rapid Method Workshop and conducts studies to identify and evaluate best available technologies
- Mid to Late 2000's – US EPA and SCCWRP conduct epidemiology studies that demonstrate a strong relationship between GI illness and Enterococcus measured by qPCR
- 2011 – SCCWRP conducts project demonstrating feasibility of producing and disseminating same-day results
- 2012 – US EPA publishes and approves rapid qPCR method for measuring Enterococcus in ambient waters
- 2014 – SCCWRP adapts EPA's qPCR method for use with droplet digital PCR
- 2022 – San Diego County gains provisional approval from US EPA and State to use ddPCR for beach monitoring

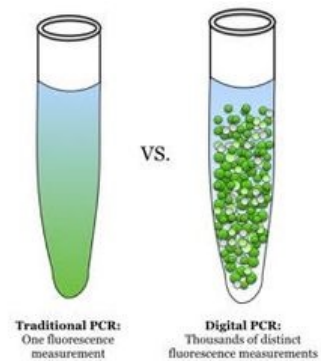
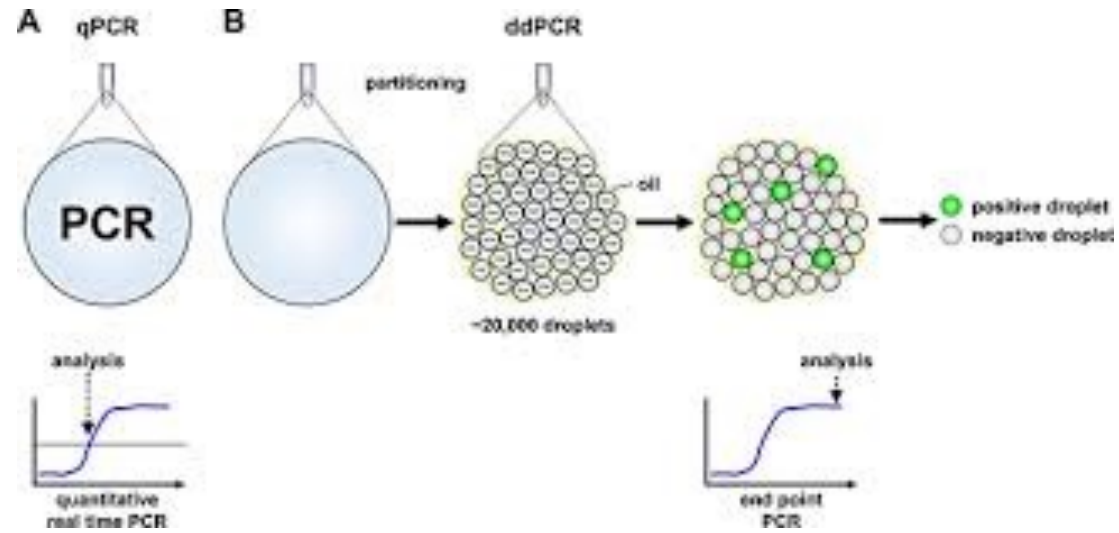
Road Map

- Growth-based vs. quantitative PCR methods
- Gaining approval for use of ddPCR at San Diego County beaches
- How has the implementation of ddPCR gone?
 - What new questions or policy issues have arisen as a result of the switch from growth-based methods to ddPCR?

Growth-based vs. Molecular Methods



qPCR vs. ddPCR



Advantages of ddPCR

- More sensitive
- Greater precision
- More resistant to environmental inhibitors
- Not reliant on reference standards for quantification

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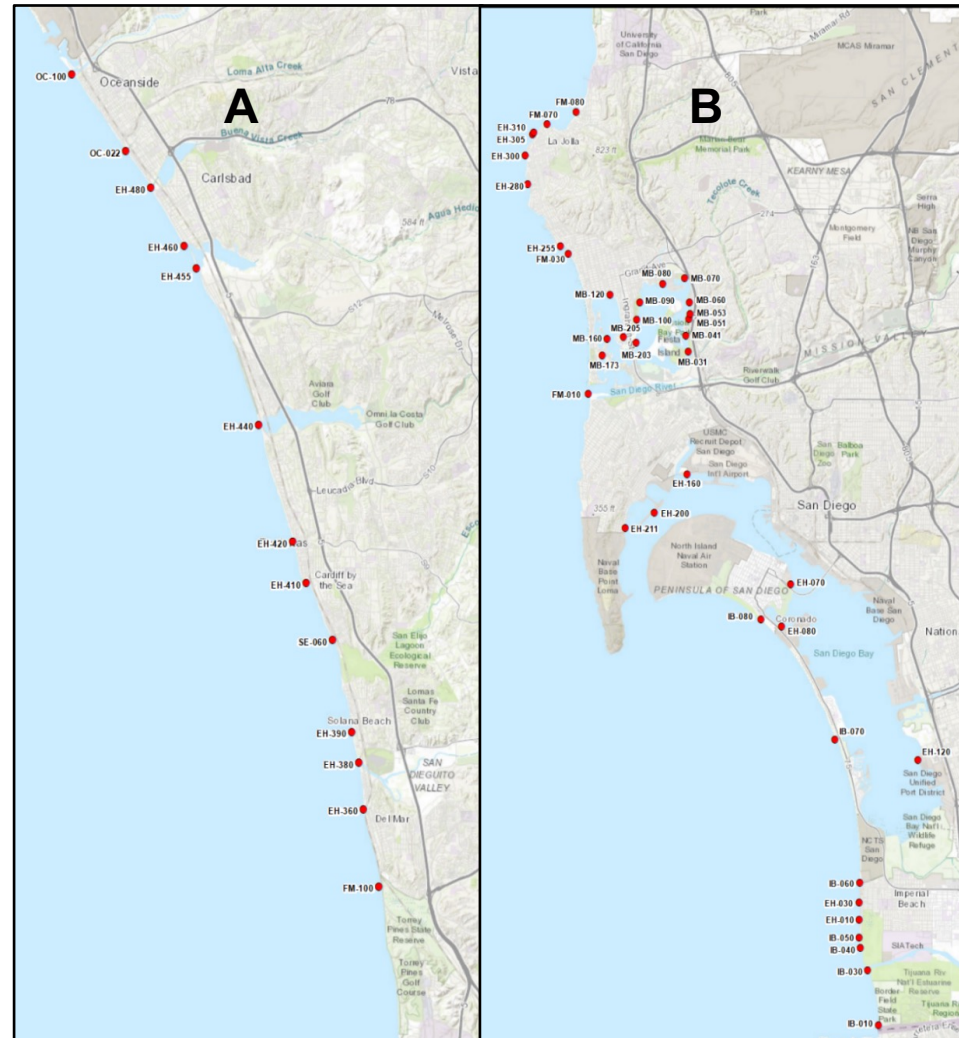
Gaining EPA Approval for a New Method Under The Clean Water Act

- Alternative Test Procedure
- Demonstrate Equivalency with an EPA-approved method (as described in *Technical Support Materials for Alternative Indicators and Methods*)
 - Provides a correlative test for gauging if a method may be considered equivalent to an existing EPA–approved method
 - Index of Agreement (IA) greater than or equal to 0.7
 - Pearson’s correlation coefficient (R-squared) greater than 0.6
 - May be used to set site specific criteria (in this case San Diego County beaches)

San Diego Study

- Collaborative effort
 - County of San Diego, Environmental Health – Study design, sample collection, Enterolert analysis,
 - CA Dept. of Public Health – qPCR analysis and ddPCR analysis
 - SCCWRP – Trained laboratories, provided guidance and technical support
- Entire San Diego County coastline
 - Over 3,000 samples
 - Samples collected at least weekly at all routine county sites (51) over two years
 - Wet and dry weather
- Side-by side comparison of ddPCR to two EPA-approved methods
 - qPCR
 - Enterolert
- Determine numerical value for ddPCR that equates to health risk similar to 104 MPN ENT/100ml

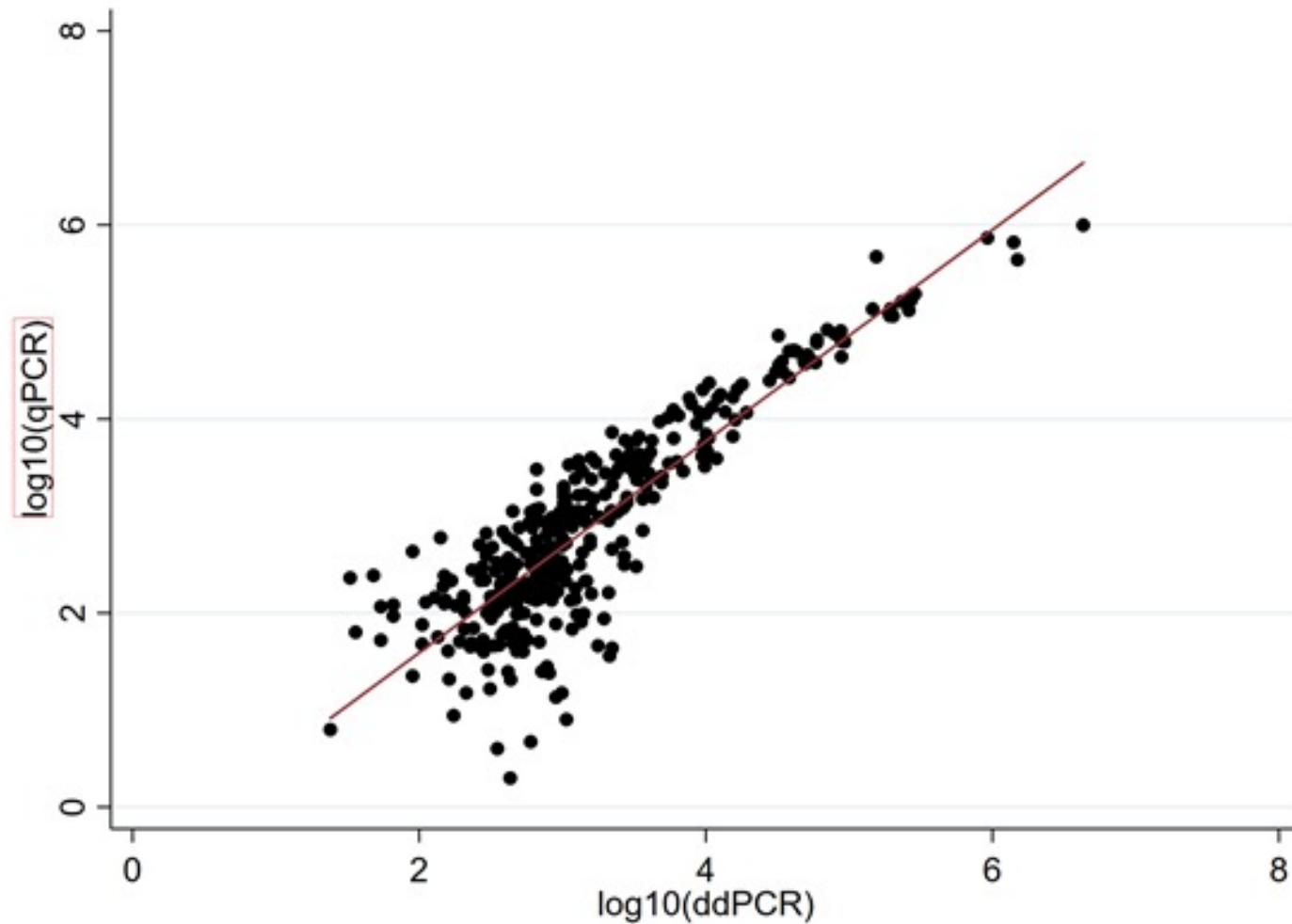
SITE MAP



Map of San Diego County coastline and designated sample locations in, (A) north coast, and (B) south coast.

Agreement Between ddPCR and qPCR

Pearson's correlation coefficient, $R\text{-squared} = 0.87$, $p < 0.005$



Study Outcomes

- ddPCR met threshold values for equivalency with Enterolert and qPCR at San Diego Beaches
 - Provisionally approved by EPA Region 9
 - County relieved of performing culture-based methods under AB 411 per SB 1395
- Water quality threshold of 1413 gene copies ENT/100ml as measured by ddPCR adopted at San Diego County beaches
- County began routine monitoring using ddPCR in May

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Method Implementation

- County lab able to collect, process and analyze samples for Enterococcus within QA/QC parameters using ddPCR
- Results available on the same day samples collected
- Greater number of beach water quality exceedances than in past years has been observed at South County beaches

Why So Many More Exceedances?

- We believe it reflects sewage coming north from Mexico
 - ddPCR more sensitive
 - Growth-based methods blind to dead or injured bacteria
- Three lines of evidence to support why we believe it is a sewage signal
 - Studies of ocean currents during South Swell events
 - Enterococcus measurements highly correlated with those of HF183 human fecal marker
 - Detection of coliphage at Imperial Beach

Policy Dilemma for Health Department

- Health Department is required to close the beach when there is a known sewage spill
- South Swell events are not technically sewage spills
 - Contamination events instead driven by ocean conditions
 - Likely presence of sewage detected by ddPCR measurements has caused the Health Department to adapt their management strategy