

## Bight '13 Study Plan

### Efficacy of qPCR Measurement of Enterococci at Southern California Beaches

Culture based methods for measuring fecal indicator bacteria (FIB) have been used to monitor the microbiological quality of recreational waters for most of the last century. Over the past decade, molecular techniques for monitoring recreational water quality have emerged as more rapid alternatives to these culture-based tests. Among these molecular methods, quantitative polymerase chain reaction (qPCR) has proven to be the most versatile, accurate and robust of these methods. Thus, in 2012 EPA published revised Recreational Water Criteria allowing the use of qPCR for measurement of *Enterococcus* for beach water quality monitoring.

As a pre-condition of using qPCR, the 2012 EPA criteria specify that studies be conducted to characterize the performance of qPCR at each beach site where it is proposed for use, as there is evidence to suggest that the method may be susceptible to site specific interferences, especially large organic molecules such as complex carbohydrates and humic acids.

Southern California beaches may be divided into two major types: Open-coast and embayment. Open coast beaches are characterized by high-energy surf and are well mixed, while those located within embayments have little or no surf and are often poorly mixed. In addition, some beaches of both types are impacted by accumulations of decaying eelgrass, kelp or other organic material of the type thought to have deleterious effects on measurements made using qPCR.

Despite the different beach types and range of conditions found in southern California, qPCR has been tested on relatively few beaches across the region. The goal of this study is to assess the efficacy of using qPCR for measurement of *Enterococcus* across the range of beach types and conditions typically found in southern California.

#### Study Questions

- How do measurements of *Enterococcus* by culture and qPCR compare between embayment and open coast beaches?
- Does the presence of kelp, eelgrass or decaying terrestrial vegetation on the beach interfere with the measurement of *Enterococcus* by qPCR?

#### Approach

Samples will be collected at selected sites and analyzed for *Enterococcus* by qPCR and culture methods at open coast and embayment beaches with or without decaying organic matter present on the beach. The correlative relationship between results from

culture vs. qPCR measurements of *Enterococcus* will be determined and the effect of interference from organic debris on the beach will be examined through amplification controls.

### **Study Design**

Thirty-five beach sites will be sampled across four counties during the summer and winter dry weather. A minimum of 50 samples per weather type will be collected at a range of open coast and embayment beaches that typically do or do not have appreciable amounts of organic material present. Samples will be collected following the routine sampling schedule for each site and analyzed in duplicate for cultivable *Enterococcus*. In addition, three replicate filters per water sample will be created within 6 hours of collection (100ml volume) and stored at -80°C until analyzed for *Enterococcus* by qPCR.

All laboratories performing qPCR as part of this study will participate in a laboratory intercalibration exercise to ensure data comparability for *Enterococcus* measurements across laboratories. Laboratories that do not meet the quality assurance standards set by the Bight '13 Microbiology Committee will be provided technical assistance. These laboratories will not be allowed to process field samples until they have demonstrated proficiency in performing qPCR to the satisfaction of the Bight '13 Microbiology Quality Assurance Subcommittee.

In addition to the tasks outlined above, a sub-set of laboratories will participate in an additional activity designed to compare reference material used to perform the qPCR assay for *Enterococcus*. Laboratory created frozen whole-cell reference material will be compared with commercially prepared genomic DNA reference material. Previous studies have shown that the type of reference material used as calibrator for qPCR measurement of *Enterococcus* is a major factor in observed differences in results when the test is performed on the same DNA extracts. Data from this experiment will be used to assist in interpreting the qPCR results from the larger study,

### **Training**

Training on sample handling and filtration will be provided by SCCWRP prior to the onset of sampling. SCCWRP will also provide training to participating agencies or their designee on measurement of *Enterococcus* by qPCR.

### **Schedule**

- Training on sample handling and filtration – July 11, 2013.
- Sample collection will commence immediately upon the completion of training and continue for two years. Laboratories previously trained in performing qPCR may start collecting samples immediately upon approval of the Study Plan by the Bight '13 Microbiology Committee.

- Training on measurement of *Enterococcus* by qPCR – August 27-29, 2013.
- Laboratory intercalibration study plan – September, 2013
- Laboratory intercalibration exercise - October, 2013
- qPCR analysis of samples will commence at each laboratory following successful completion of the laboratory intercalibration exercise.

### Data Reporting and Analysis

SCCWRP will compile and conduct initial analysis of study data. Pre-prepared field and data reporting sheets will be provided to each participating agency/laboratory as needed for the field, laboratory intercalibration and sample analysis components of the study. Electronic field site occupation reporting devices will be provided if available.

**Table 1. Sampling Sites**

<b>Embayment or Open Coast</b>	<b>Kelp or Eelgrass</b>	<b>Monitoring Agency</b>	<b>Station Name</b>	<b>Location</b>
Open	no	OCSD	6N	Huntington Bch.
Open	no	OCSD	9N	Huntington Bch
Open	no	OCSD	OSB02	Seal Beach
Open	no	OCSD	BGCD	Little Corona
Open	no	OCSD	WFCD	Pelican Hill
Open	no	OCSD	MDCD	Reef Point
Open	no	OCPHL	POCHED (S-15)	Poche Beach
Open	no	CO	San Luis Rey River	Oceanside
Open	no	DEH	Moonlight Beach	Encinitas
Open	no	CLA	SMB BC-1	Playa del Rey
Open	yes	OCSD	0	Huntington Bch.
Open	yes	OCSD	3N	Huntington Bch
Open	yes	OCPHL	PICOD (S-17)	Doheny

Open	yes	CSD	Bermuda Avenue	Ocean Beach
Open	yes	CLA	SMB-7-4	Abalone Cove
Open	yes	CLA	SMB-7-1	Malaga Cove
Open	yes	CLA	SMB 1-10	Dan Blocker
Open	yes	CLA	SMB MC-2	Surfrider
Open	yes	CLA	SMB 1-18	Topanga
Open	yes	CLA	SMB 3-3	Santa Monica
Open	yes	CSD	Stub Jetty (south side)	Ocean Beach
Open	yes	CSD	Tijuana River Mouth	Imperial
Embayment	no	OCPHL	BHH 15	Huntington Harbor
Embayment	no	OCPHL	MHH10	Newport Bay
Embayment	no	OCPHL	BNB33	Newport Bay
Embayment	no	OCPHL	BNB24E	Newport Bay
Embayment	no	LBPH	B22	Long Beach
Embayment	no	LBPH	B31	56th Place/Bayside
Embayment	no	CLA	MdRH-2 (S9)	Marina del Rey
Embayment	no	VCEH	Kiddie Beach	Oxnard
Embayment	yes	CSD	Visitor's Center	Mission Bay
Embayment	yes	OCSD	BNB28	Newport Bay
Embayment	yes	OCSD	BNB20	Newport Bay
Embayment	yes	CSD	Tecolote	Mission Bay
Embayment	yes	CSD	Cudahy	Mission Bay

Embayment	yes	VCEH	Hobie Beach	Oxnard
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