Work/Quality Assurance Project Plan:

Rapid Method Demonstration Project at Four New Jersey Marine Beaches using Real Time Polymerase Chain Reaction (qPCR)

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TABLE OF CONTENTS

List of Tables and Figures

1.0 Project Name:

Rapid Methods Demonstration Project at Four New Jersey Marine Beaches using Real Time Polymerase Chain Reaction (qPCR)

2.0 Requested By:

USEPA Region 2 and Water Monitoring and Standards, Bureau of Marine Water Monitoring, NJDEP

3.0 Date of Request:

June 21, 2011

4.0 Date of Sampling Initiation:

June 20 & 27, 2011 – Exercise (No Data Reported)
July 5th, 2011 – Official Project Start Date.

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7.0 Project Description

This demonstration project is a culmination of studies conducted in the summers of 2007-2010. This project will provide data using qPCR results on a real time basis. Advisories will be issued if needed. This demonstration project will include sampling at four marine bathing beaches in the Toms River area of Ocean County and will provide *Enterococcus* data for beach management decisions on the same day that samples are collected. This demonstration project will also test the ability to collect, analyze and report bacteriological sample using a more rapid method for determination of *Enterococcus* in marine bathing beach samples.

7.1 Project History

In 2007, 20 ocean and bays were sampled throughout Ocean and Monmouth Counties, New Jersey. Comparison of *Enterococcus* over a wide range of target concentrations was evaluated using qPCR and conventional microbiological analyses.

In 2008, the study design included intensive sampling at two sampling areas, Myron/Wilson Bay (Memorial Park), Neptune City, Monmouth County and Central Avenue, Island Heights, Ocean County. In this study, split samples were collected along an 80 M transect for spatial variability assessments. Also, sampling was performed in the morning, afternoon, as well as 24 hours later to measure temporal changes in qPCR results as compared to conventional membrane filtration analysis for *Enterococcus*. Variability from wet weather influences was also evaluated as part of this study.

In 2009, the test design included a comparison of *Enterococcus* concentrations from 10 sites in Monmouth and Ocean County New Jersey. Many of these sites were originally sampled in 2007. Also, USEPA analyzed split samples using 2 different qPCR platforms, ABI 7500 and the Cepheid Smart Cycler to determine if different qPCR platforms manufactured by different companies deliver similar results.

In 2010, a total of 10 stations that were sampled in 2009 were revisited. Each county sampled five sites every other week over a nine week period. Membrane filtration was conducted by OCUA for Ocean County samples and USEPA Region 2 for the samples collected by MCHD. Subsamples from each sample aliquot collected were also analyzed for *Enterococcus* via qPCR by USEPA Region 2. A duplicate set of samples was also filtered for qPCR from all stations from both counties and archived for use by NJDEP, Leeds Points, NJ.

MF results from the 2009 and 2010 studies indicated that a large portion of the samples contained lower Enterococcus concentrations than anticipated based on historical results. This trend severely impacted the objectives of the study to evaluate statistical differences between laboratories and/or thermal cyclers. A

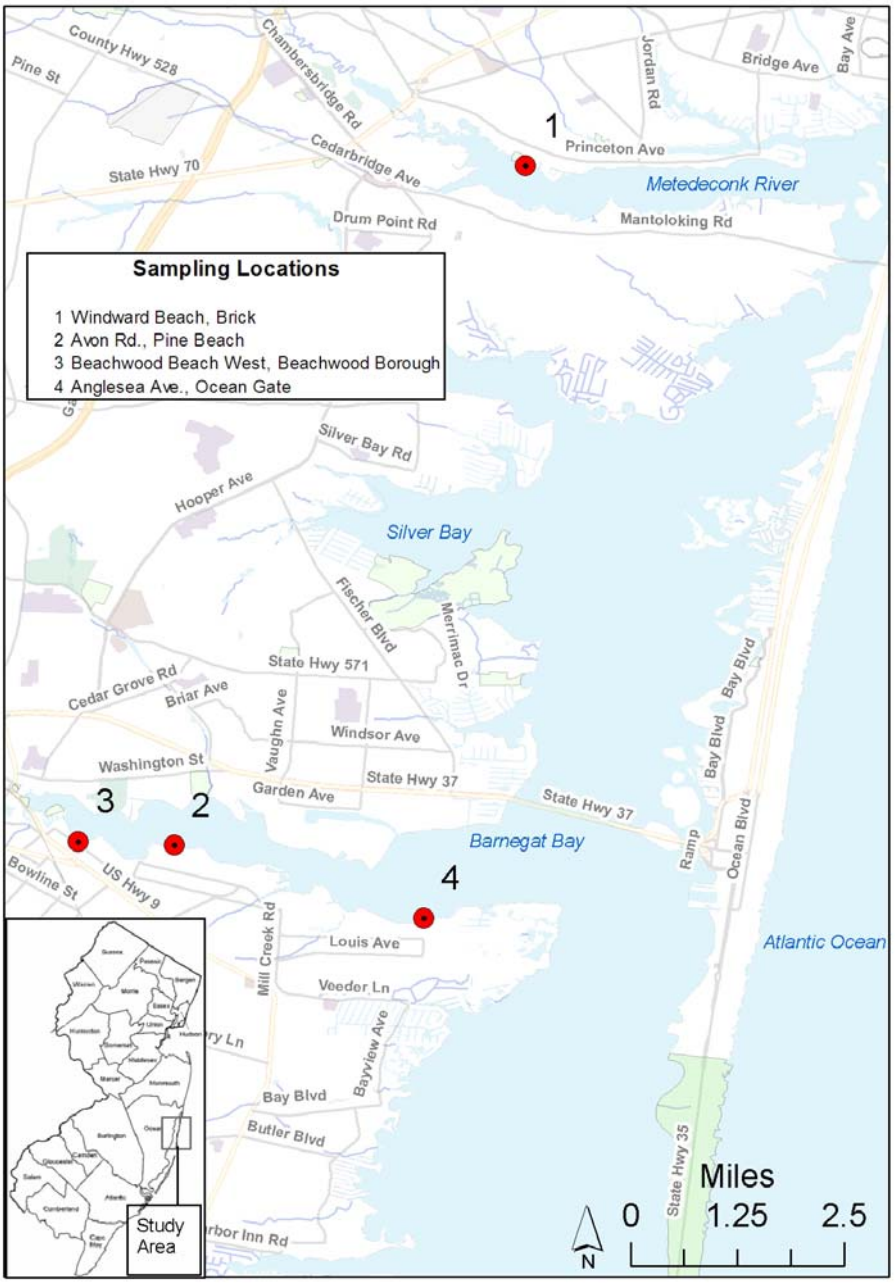

Figure 1. Map of Beach Sampling Stations Sampled as part of the 2011 qPCR Demonstration Project, July - August 2011

data summary for the 2009/2010 sampling efforts will be available in December 2011.

7.2 2011 Project Overview

Samples will be collected in a sterile 500 mL bottle at each station by Ocean County Health Department at four locations in Ocean County, NJ (Figure 1). Split sample aliquots will be used from each bottle for both qPCR and membrane filtration analysis. Samples will be collected in lower thigh to chest deep water, approximately 0.3 meters from the surface. Samples will be transported to the OCUA laboratory on ice and will be analyzed for *Enterococcus* sp. using the traditional membrane filtration (MF) technique by OCUA. EPA will perform the qPCR analysis. Results of MF and qPCR will be reported on the www.*NJBeaches.org* website as soon as possible after data collection. Based on data from the 2007 and 2008 studies, a site specific action threshold of 120 Calibrator Cell Equivalents (CCE) per 100 mL of sample was calculated for enterococci using qPCR. If this value is exceeded in the morning samples, swimming advisories will be issued and a confirmation qPCR analysis on new samples collected in the afternoon will be conducted.

8.0 Project Definition and Background

Previous health and epidemiological studies by USEPA have demonstrated that densities of the bacterial genus *Enterococcus* in both marine and freshwater samples are directly correlated with gastroenteritis illness rates in swimmers exposed to these waters (Cabelli 1982, Dufour 1984). USEPA requires that recreational waters across the United States be monitored routinely for *Enterococcus* spp. and /or *Escherichia coli*. While neither of these organisms is pathogenic, both are considered to be surrogates of the presence of bacterial and viral pathogens found in fecal material. Currently, approved methods for measuring concentrations of *Enterococcus* and *E. coli* in recreational waters include membrane filtration, Most Probable Number (Multiple Tube Fermentation (MTF) techniques and DST® (Defined Substrate Technology) tests. Although many of these methods have been used for decades, results are generally not available for at least 24 hours. Due to the fluctuating nature of microbial contamination, this delay makes it difficult for beach management officials to make decisions regarding beach closures and advisories. At best, decisions are made using one day old information; or a decision regarding safe beach usage is not made until after a confirmation test which may be up to 72 hours after the initial sample was collected. USEPA (2005) found that changes in water quality conditions during this delay can frequently lead to notifications to the public that are not fully protective of bathing beach users health. Because microbial water quality can change rapidly (Boehm et. al., 2002), guidelines based on indicator organisms that require 24 hours to develop, may result in both unnecessary beach closings or exposure of swimmers to poor microbial water quality. A recent study estimates that up to 40% of beach closures are in error (Kim and Grant, 2004).

There is a need for more rapid indicators of recreational water quality. Congress passed an amendment to the Clean Water Act, the Beaches Environmental Assessment and Coastal Health (BEACH) Act. A major objective of the BEACH Act required the U.S. EPA to conduct research to provide the support of new criteria for recreational water. The use of qPCR assays have shown promise as an alternative technology to monitor microbial water quality at recreational beaches which have been examined at both freshwater and marine recreational areas (Haugland, et. al. 2004; Wade, et. al, 2005; Morrison, et. al. 2008; and Ferretti, et. al 2011).

Primer sets and probes are commercially available for the specific detection of *Enterococcus* spp. using qPCR. Protocols for qPCR are now available to quantify indicator bacteria in recreational waters in approximately 4 hours after sample receipt. Because these methods provide a faster assessment of water quality, they have the potential to improve decision making for personnel involved in beach management decisions.

In 2007, USEPA Region 2, New Jersey Department of Environmental Protection (NJDEP), and Ocean and Monmouth County Health Departments collaborated on a comparison study using qPCR and conventional microbiology methods at 20 beaches in Ocean and Monmouth Counties. Ocean and bay samples with varying levels (based on historical data) of expected microbial densities were sampled 10 times between June 18 and August 20, 2007. Cell densities of *Enterococcus*, were determined by qPCR, as well as two conventional 24 hour test methods (Membrane Filtration and Enterolert). Over 1000 samples were analyzed and qPCR concentrations generally increased or decreased in relation to the corresponding MF or Enterolert analysis. Regression analysis of these results showed a significant positive correlation between qPCR and MF/Enterolert methods with an overall correlation coefficient of 0.71. Also, similar real time demonstration projects at marine sites in Orange County California and freshwater sites in Racine Wisconsin were successfully completed recently.

9. Project Schedule

Final QAPP	July 1, 2011
Field Sampling Begins*	July 5, 2011
Field Sampling Ends	August 29, 2011
Analysis/qPCR and EPA 1600	Within 6 Hr Holding Time
Draft Report	November 2011
Final Report	February 2012

*A two event practice run will performed during the last 2 weeks of June 2010

10. Quality Objectives and Criteria

10.1 Precision

Variability is inherent in marine waters analyzed for *Enterococcus*. Within sample and within station variability have been documented previously (EMPACT, 2005, EPA 2008, Ferretti et al. 2010). All sample aliquots will be filtered from one sample bottle. It is important to rehomogenize the sample bottle between every aliquot filtered.

10.2 Bias

For the qPCR analysis, a filtration blank will be prepared at the beginning and end of sample filtration. Blank data will be evaluated to determine the potential for contamination. A salmon DNA Sample Processing Control will be included with each test sample to test for proper DNA amplification.

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10.3 Representativeness

The sampling methodology is designed to ensure that the samples are representative of the area where they are collected. The stations were selected because historical qPCR data exists and the locations are situated to minimize transport time to the laboratory.

10.4 Comparability

Monitoring of bacterial concentrations has been performed for many years as part of the CCMP at the selected sampling stations. qPCR data is available from all of the sampling areas from our 2007 qPCR project. USEPA Method 1600 is a standard method for *Enterococcus* measurement. USEPA qPCR methodology was developed from Haugland (2005) and outlined in USEPA SOP 10.1 and USEPA Method A.

10.5 Completeness

Ideally, samples should be collected from all stations over the prescribed study period. It is anticipated that completion of 80% of the sampling would be adequate to meet the objectives of this study. Unforeseen obstacles such as severe weather or a priority event at the Ocean County Health Departments may prevent sampling for a particular week.

10.6 Sensitivity

The analytical techniques proposed for this study should demonstrate adequate sensitivity to meet the objectives of the project.

11. Documentation and Records

Field and laboratory data will be sent by Ocean County Health Departments to the USEPA Region 2 Project Officer at the end of the project. qPCR data will be maintained by USEPA Region 2. All logbooks, field and lab notebooks, reports, data sheets, and quality control information must be maintained on file by all participating organizations until a final project report is issued.

12. Experimental Design

12.1 Depth

Samples will be collected in an area of depth of approximately lower thigh to chest deep water. Samples will be collected by placing sterilized containers 8 to 12 inches (approx 0.3 M) below the surface of the water and removing the bottle tops to collect the sample.

Rationale:
Of all of the variables evaluated in the EMPACT study, the greatest single determinant of microbial indicator level was found to be the depth zone. Bacterial densities became substantially lower as one moved from ankle-deep to knee deep to chest-deep water.

Sampling in lower thigh to chest deep water would seem to offer a reasonable, but still conservative approach to estimating water quality. Sampling from 8 to 12 inches below the surface is justified based on exposure considerations. The EMPACT study did not find any differences in bacterial concentration throughout the vertical profile of the water column as long as the overall depth of the sampling area was consistent (i.e., lower thigh to chest deep water).

12.3 Sampling Time

Samples must be collected in the morning as early as possible and preferably by 8:00 am to allow for timely same day reporting of results. If confirmation samples for qPCR are needed in the afternoon, a sampling time of no later than 1:00 pm should be observed.

Rationale:
An earlier sampling time will allow for a same day confirmation sampling in the event the morning qPCR result indicated unacceptable bacterial concentrations. This is to provide Information to the public in a timely manner.

13. Sampling Methods and Procedures

There will be a total of four sampling stations that will be sampled beginning July 5 and continuing weekly until August 29, 2011 (Figure 1, Tables 1 & 2).

Samples will be collected directly in a sterile HDPE 500 mL containers following procedures outlined in NJDEP, Cooperative Coastal Monitoring Program (CCMP), Quality Assurance Project Plan, FY11/FY12, Section 12.1, Sample Collection; in Chapter IX (Public Recreational bathing) of the State Sanitary Code, N.J.A.C. 8:26-1 et seq. (amended April 2004) and described briefly here.

Samples will be collected in a sterile container in an area with a stabilized water depth between the sampler's lower thighs and chest. The sample container shall

be placed approximately 8-12 inches below the water surface with the lid still attached. With the collector's arms extended to the front, the container shall be held near its base and downward at a 45-degree angle. The cap

Table 1. Proposed Sampling Schedule, 2011 qPCR Demonstration Project

Tuesday, July 5	Sampling Event 1
Monday July 11	Sampling Event 2
Monday July 18	Sampling Event 3
Monday, July 25	Sampling Event 4
Monday August1	Sampling Event 5
Monday August8	Sampling Event 6
Monday August 15	Sampling Event 7
Monday August 22	Sampling Event 8
Monday August 29	Sampling Event 9

Table 2. Sampling Stations, 2011 qPCR Demonstration Project at Four Marine Bathing Beaches in Ocean County New Jersey Beach

Sampling Area / Station #	Location
Windward Beach, CCMPOC0103	Brick Township
Avon Road, CCMPOC0017	Pine Beach
Beachwood Beach, CCMPOC0116	Beachwood Beach
Anglesea, CCMPOC0140	Ocean Gate

shall be removed and the container filled in one slow sweeping motion (downward or horizontally, not upward). The mouth of the container shall be kept ahead of the collector's hand and the container recapped while it is still submerged. The cap shall remain submerged during sample collection and care shall be taken not to touch the inner surface of the cap. Samples collected for this project shall not be taken with sampling poles.

Samples must be kept on ice during transport and held at 1-4^{o}C at the laboratory if filtration is not performed immediately. Do not freeze the water samples. Samples will be transported to the laboratory and filtered for qPCR preparation as soon as possible after collection. The filters for the qPCR analysis must be processed immediately, otherwise they will be frozen at -20 to -70^{o}C. USEPA personnel will perform qPCR analysis at the OCUA Laboratory in Bayville, NJ. This location was selected to allow for a more central location for sample analysis. OCHD personnel will collect the initial samples used each week for the study. If confirmation tests are required for qPCR analysis, USEPA Region 2 or OCHD personnel will collect these samples. It is anticipated that afternoon samples will be collected to coincide with completion of the qPCR run on the initial sample to save time in case results indicate that a confirmation test is

needed. If initial qPCR results are less than the threshold of 120 CCE/100 mL is indicated, the confirmation samples will not be used.

Time and date of sample collection, location, air/water temperature, wind direction, rainfall, and estimated number of people on the beach will be recorded on the Chain of Custody form.

Training and/or certifications necessary to conduct field and laboratory analysis by all participating personnel will be performed in accordance with each organizations Quality Management Plan.

14. Sample Handling and Custody

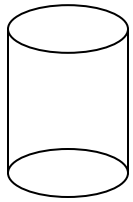
Chain of custody forms are required for this project. NJDEPE, Field Sampling Procedures Manual, Trenton, NJ (August 2005) and the requirements of the NJDEP's CCMP QAPP must be followed for all field procedures. Ocean County Health Department has an approved Standard Operating Procedure (SOP) regarding sample collection, receipt and/or preservation for USEPA Method 1600. USEPA qPCR SOP 10.1 contains sample preparation and handling procedures for the qPCR samples.

14.1 Sample Containers, Preservation, Holding Times

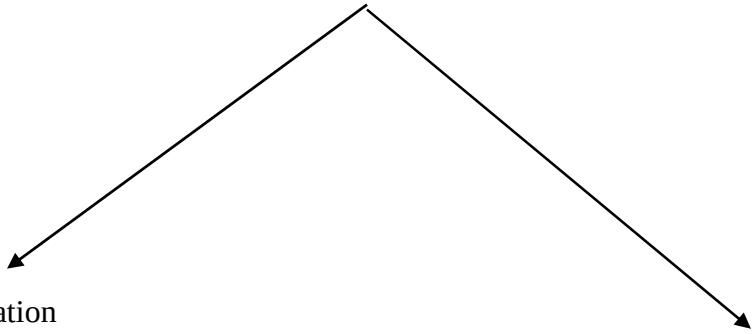
The sample containers shall be sterilized 500 mL HDPE plastic containers. The water samples for USEPA Method 1600 and USEPA qPCR must be subsampled from the same bottle. Shake the sample bottle frequently to keep bacteria well mixed in between each filtration. The holding time for the *Enterococcus* MF test will be six hours. The MF procedure will be USEPA Method 1600 and performed in accordance with the SOPs established by OCUA. Samples for qPCR must be filtered within six hours and analyzed within 8 hours of sampling. Analysis for both qPCR and membrane filtration is anticipated to be filtered much sooner to allow for more timely reporting of the data for beach usage decisions. Archive samples for qPCR should be filtered within 6 hours of collection and frozen at -20 or -70 ^{o}C until analysis. The frozen filter papers may be stored for up to one year.

14.2 Sample Filtration

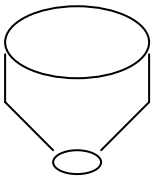
14.2.1 USEPA Method 1600 MF Sample Filtration



500 mL Sample Bottle (HDPE)
Shake 25 times to homogenize
Re-shake between every sample aliquot used



Membrane Filtration
(OCUA)



qPCR

1. Perform qPCR Filter blank at beginning of sample using Nuclease Free sterile water

2. Filter for PCR sample
Total 3 replicates per sampling station

3. Perform PCR Filter blank at end of sample filtration using Nuclease Free sterile water

4. Assign unique ID number and place on Filtration Log and each 2 mL beaded tube before analysis. Freeze third filter of each sample for archive.

Figure 2. Schematic of sample filtering for 2011 qPCR Demonstration Project

Filter paper for the PCR analysis should be from GE Water & Process Technologies (1-800-444-8212) or equivalent. The filters are a polycarbonate material with a 0.4 micron size and 47 mm diameter (Cat.

Procedures for *Enterococcus* analysis using USEPA Method 1600 as described in the approved method protocol and laboratory method SOP will be followed by OCUA.

14.2.2 qPCR Sample Filtration
No. K04CP04700). Disposable filter funnels from Nalgene must be used for all samples filtered for PCR (Nalgene 145-0020).

The membrane that comes with the disposable Nalgene brand or equivalent filter must be removed before use and replaced with the polycarbonate filter. Use one disposable filter funnel for each sample replicate filtered (Figure 2). One water sample filtration blank in duplicate must be prepared at the beginning of filtering for the qPCR analysis using DNA/RNA free water (Figure 2). Another filtration blank should be prepared at the end of the filtering of the test samples
The qPCR sample filtration should be documented on the 2011 qPCR Beach Study Filtration Log (Table 3). One log sheet should be filled out for each sampling event. The ID field of this log should be sequentially numbered. Every test sample and blank will be sequentially numbered on the top of the 2 mL beaded tube with the following designation: X-XX (X = Week number and XX = sequential sample number from 1 to12). The initial and final blanks will be designated a X-Y (X = Week Number and Y = Letter A for initial blank and B for final blank). These numbers must be entered onto the Filtration Log as well (Table 3).

<u>14.2.3 USEPA qPCR General Sample Filtering Procedures</u>

Shake the sample bottle vigorously 25 times to distribute the bacteria uniformly and measure a 50 mL sample into a sterilized 50 mL centrifuge tube (the original filter that comes with the funnel should have been discarded and replaced with a polycarbonate filter using sterile forceps). Filter 50 mL of water sample. **Record the volume of water filtered using Figure 3 if not 50 mLs.** After filtering the sample, rinse the sides of the funnel with 20-30 mL of sterile RNA/DNA free water and continue filtration until all liquid has been pulled through the filter. The 500mL sample bottle must be shaken every time a 50 mL aliquot is dispensed. A 50 mL tube rack can be set up with 14 50 mL centrifuge tubes (2 blanks and 4 test samples in triplicate) immediately before filtration. A new filter cup must be used for each sample replicate and blank.

Turn off the vacuum and remove the funnel from the filter base using a sterile set of forceps. On the filtration unit cap or a clean and sterile Petri dish lid or base, fold the filter into a cylinder shape with the sample side

Table 3. qPCR Filtration Log

ID	Blank/ Sample Rep	STATION	REP	FILTER TIME	SAMP TIME	VOLUME FILTERED
	Blank Initial	**NA**	**Blank$_i$**		**NA**	**50 mLs**
	A	**Station 1**	**A**			**50 mLs**
	B	**Station 1**	**B**			**50 mLs**
	C	**Station 1 Arch**	**C**			**50 mLs**
	A	**Station 2**	**A**			**50 mLs**
	B	**Station 2**	**B**			**50 mLs**
	C	**Station 2 Arch**	**C**			**50 mLs**
	A	**Station3**	**A**			**50 mLs**
	B	**Station 3**	**B**			**50 mLs**
	C	**Station 3 Arch**	**C**			**50 mLs**
	A	**Station 4**	**A**			**50 mLs**
	B	**Station 4**	**B**			**50 mLs**
	C	**Station 4 Arch**	**C**			**50 mLs**
	Blank Final	**Station 1**	**Blank$_f$**		**NA**	**50 mLs**
AFTERNOON CONFIRMATION SAMPLES						
	Blank Initial	**NA**	**Blank$_i$**		**NA**	**50 mLs**
	A	**Station 1**	**A**			**50 mLs**
	B	**Station 1**	**B**			**50 mLs**
	C	**Station 1 Arch**	**C**			**50 mLs**
	A	**Station 2**	**A**			**50 mLs**
	B	**Station 2**	**B**			**50 mLs**
	C	**Station 2 Arch**	**C**			**50 mLs**
	A	**Station3**	**A**			**50 mLs**
	B	**Station 3**	**B**			**50 mLs**
	C	**Station 3 Arch**	**C**			**50 mLs**
	A	**Station 4**	**A**			**50 mLs**
	B	**Station 4**	**B**			**50 mLs**
	C	**Station 4 Arch**	**C**			**50 mLs**
	Blank Final	**Station 1**	**Blank$_f$**		**NA**	**50 mLs**

facing inward with sterile forceps (flame the tips in-between samples or use disposable sterile forceps), being careful to handle the filter only on the edges, where the filter has not been exposed to the sample. It is best to use 2 sets of sterile forceps. Once the filter is placed on the turned over filtration cup lid or Petri dish top or bottom, fold the filter paper in half, using one of the forceps to hold the filter down. Repeat folding in half 2 more times and insert the folded filter into the labeled extraction tube with glass beads. A new Petri dish or filter cup lid should be used for each unique filter. Label the extraction tube with glass beads with a unique ID that was explained in the section 14.2.2. Cap the extraction tub. Samples for qPCR will be run in duplicate for each station. The third sample will be archived and will be frozen at -20^{o}C to -70 ^{o}C (Table 4).

15. Laboratory Analysis/Analytical Methods

15.1 *Enterococcus* using Membrane Filtration

Viable enterococci will be enumerated by USEPA Method 1600 by OCUA. A volume of 10 - 100 mL from each replicate will be filtered on 47-mm diameter, 0.45 micron pore size membrane filters. The filters will be incubated on plates of mEI agar for 24 ± 2h at 41 ± 0.5 degrees centigrade. Colony counts from the most appropriate dilution will be reported unless they exceed 200, in which case counts from one square will be counted and a multiplier will be used to express enterococci as CFU per 100 mL of water. Additional quality control procedures are identified in the OCUA SOPs.

15.2 *Enterococcus* using qPCR

Total *Enterococcus* via qPCR will be performed using a Cepheid Smart Cycler Thermal Cycler unit by USEPA using USEPA Region 2 SOP Bio 10.1 (based on Haugland et. al. 2005 and EPA Method A). Primers and probes will be purchased from ABI, Inc. or other appropriate vendor. This method is initiated by filtering a water sample through a membrane filter (Figure 2). The membrane filter containing the bacterial cells and DNA is placed in a microcentrifuge tube with glass beads and buffer. The microcentrifuge tube with glass beads and buffer, is agitated to extract the DNA into solution. The supernatant is used for PCR amplification and detection of target sequences using OmniMix PCR reagent or equivalent and the TaqMan probe system.

16. Quality Control

A systematic procedure to document all phases of the qPCR, and MF analysis will be used to address equipment maintenance, reagent quality,

and test quality assurance. All QC activities related to balance and pipette calibrations, incubator temperature monitoring, preparation of media, sterilization procedures and field measurements must be followed in accordance with each laboratory SOPs. Method specific QC requirements are outlined below for each method.

16.1 QC Practices: qPCR, USEPA SOP 10.1

Media Sterility Check: Each batch of media (BHIA/BHIB) will be checked for sterility by incubating one plate and observed for growth. Absence of growth indicates media sterility.

Method Blank: A 50 mL volume of sterile DNA/RNA free water will be filtered at the beginning and end of sample filtration. If the method blanks are contaminated, then the samples will be rerun or data may be qualified.

Positive Controls: The laboratory will analyze positive controls to ensure that the method is performing properly. Calibrator samples, or samples with known concentrations of *Enterococcus faecalis* will be analyzed on a weekly basis. If a positive control fails to exhibit the correct fluorescent growth curve response, the reagents will be checked and the reanalyzed. If positive controls fail again, new calibrator samples will be prepared and reanalyzed.

No Template Controls (NTC): An NTC will be performed on each lot of PCR Master Mix reagents to document that they are not contaminated. If greater than 1/3 of the NTC reactions for a PCR master mix exhibit true positive log amplification traces with Ct values below 45, or if any one NTC reaction has a Ct value lower than 35, then the analyses will be repeated with new Master Mix stock solutions.

Sample Processing Control (SPC): An SPC using salmon DNA will be run in conjunction with each test sample to document proper amplification of DNA sequences.

16.2 QC Practices: Membrane Filtration, USEPA Method 1600

Media Sterility Check: Inoculate a mEI plate with a culture of *Enterococcus faecalis*, and another mEI plate with a culture of a culture of *E. coli*. Incubate at 41°C ± 0.5EC for 24 hours ±2 hours and check for growth. Only the enterococci culture should produce colonies with a blue halo. If sterility or performance testing fails, the prepared media is discarded.

Table 4. Sample Handling Requirements and Analytical Requirements, USEPA Method 1600, and qPCR Microbial Water Quality Comparison

Analyte[4]	# Samples[2]	Sample Volume	Container	Preservation	Holding Time	Method	Reporting Units	Laboratory
Enterococcus, MF	36	500mls *	Sterile HDPE	None	6 Hours	EPA Method 1600	CFU/100 mL	OCUA
Enterococcus, qPCR	72	*Subsampled from Enterococcus bottle	N/A	Freeze Filter - 20 ^{o}C to -70 ^{o}C	6 Hours to filter	EPA SOP Bio 10.1	CCE/100 mL	USEPA Region 2
Water/Air Temperature	9	NA	NA	NA	Field Measurement	Thermometer	0.2 ^{o}C	OCHD.

[1]*Sample volume for qPCR should be taken from the sample container used for MF analysis.*
[2]*Number of samples does not include samples for QC, confirmation, or archive samples*

Method Blank: Before and after each sampling event, filter sterile phosphate buffered saline rinse water (PBS) through filter onto a membrane filter, and aseptically transfer to an m-Endo plate. Incubate for 24 hours ± 2 hours at 35°C ± 0.5°C. No colonies should grow on both sets of plates. If a method blank is contaminated, then the samples will be evaluated and qualified accordingly.

17. Instrument/Equipment Calibration, Maintenance and Frequency

All laboratory equipment will be tested, calibrated and maintained in accordance with existing SOPs, equipment manufacturers recommendations, and method guidance documents. Laboratory equipment will be calibrated as recommended by the manufacturer, approved SOP, and/or USEPA Method 1600, and USEPA Region 2 SOP Bio 10.1.

18. Data Management

Field results and notes will be recorded in bound field notebooks in indelible ink and/or triplicate copy Chain of Custody/Field Data Sheets which will be retained by the respective project officers for Ocean County and EPA Region 2 until a final report is issued. Laboratory data will be transmitted in electronic format to the project officer at USEPA Region 2 and NJDEP (Table 5). Timely reporting of qPCR data is critical for the project. qPCR information will be communicated to Virginia Loftin or assignee at NJDEP for posting and Matt Csik and John Protonetis from OCHD. The data will be reviewed by NJDEP and then posted on the website www.njbeaches.org. Advisories may be issued based in initial qPCR results and a qPCR confirmation test will be performed the same day of any result above the established qPCR advisory threshold of 120 CCE/100 mL. qPCR samples will be run in duplicate for each sampling station. Both results must exceed 120 CCE/100 mL to be considered an exceedance of the advisory threshold. The average of both qPCR duplicates will be used as the reported value. The reporting limit for qPCR is 5 CCE/100 mL and the reporting limit for MF will be 10 CFU/100 mL if a 10 mL sample is used. The membrane filtration data and other components that are in place as part of the CCMP will ultimately be used for determination of beach closings. The flow chart to be used for this project is included as Figure 4. Attachment A lists all of the key personnel involved in the data generation and usage in this study.

19. Data Review, Verification and Validation

Data generated during this project will be validated internally according to the SOP's from each laboratory. The validated data from all laboratories

will be checked by the project officer, with technical assistance from the QA manager within each laboratory if required.

Any limitations on the use of data, as indicated by data qualifiers added during the validation process will be included in the project reports, and noted on the data summary spreadsheet maintained by the project officer. If blanks show contamination, the data may still be usable, but its impact on the results must be documented.

Copies of chain of custody forms and qPCR Filtration Logs (Figure 3) will be maintained by the USEPA Project Manager. The original copies of the chains of custody will be maintained by the field personnel responsible for collecting the samples.

20. Corrective Actions

20.1 Field

Sampling will be once weekly over the proposed sampling period and repeated on the same day if the qPCR threshold is >120 CCE/100 mL or > 104 CCE/100 mL for membrane filtration results. This date may be rescheduled or postponed if unsafe sampling conditions exist or if representative samples cannot be obtained. Any changes in sampling locations, time of sampling and variations in sampling procedures or Laboratory protocol must be agreed upon by all collaborating government entities involved in this project.

20.2 Laboratory

All laboratories should follow the established Corrective Action procedures which are in existing SOPs and/or Laboratory Quality Management Plans.

20.3 Reports:

The final report will be prepared by USEPA Region 2. A draft report will be distributed for comment among all participants. A final report will be issued and will be written using the following format:
Introduction/Background/Purpose/Objectives
Methods and Materials
Results and Discussion
Conclusions and Recommendations

20.4 Secondary Uses of the Data

Secondary uses of data for development of national or site specific criteria are possible. Public outreach and education through brochures, fact sheets, the njbeaches.org website is also an objective of this project. Also, technical training of other government entities on the use of the qPCR instrument is planned.

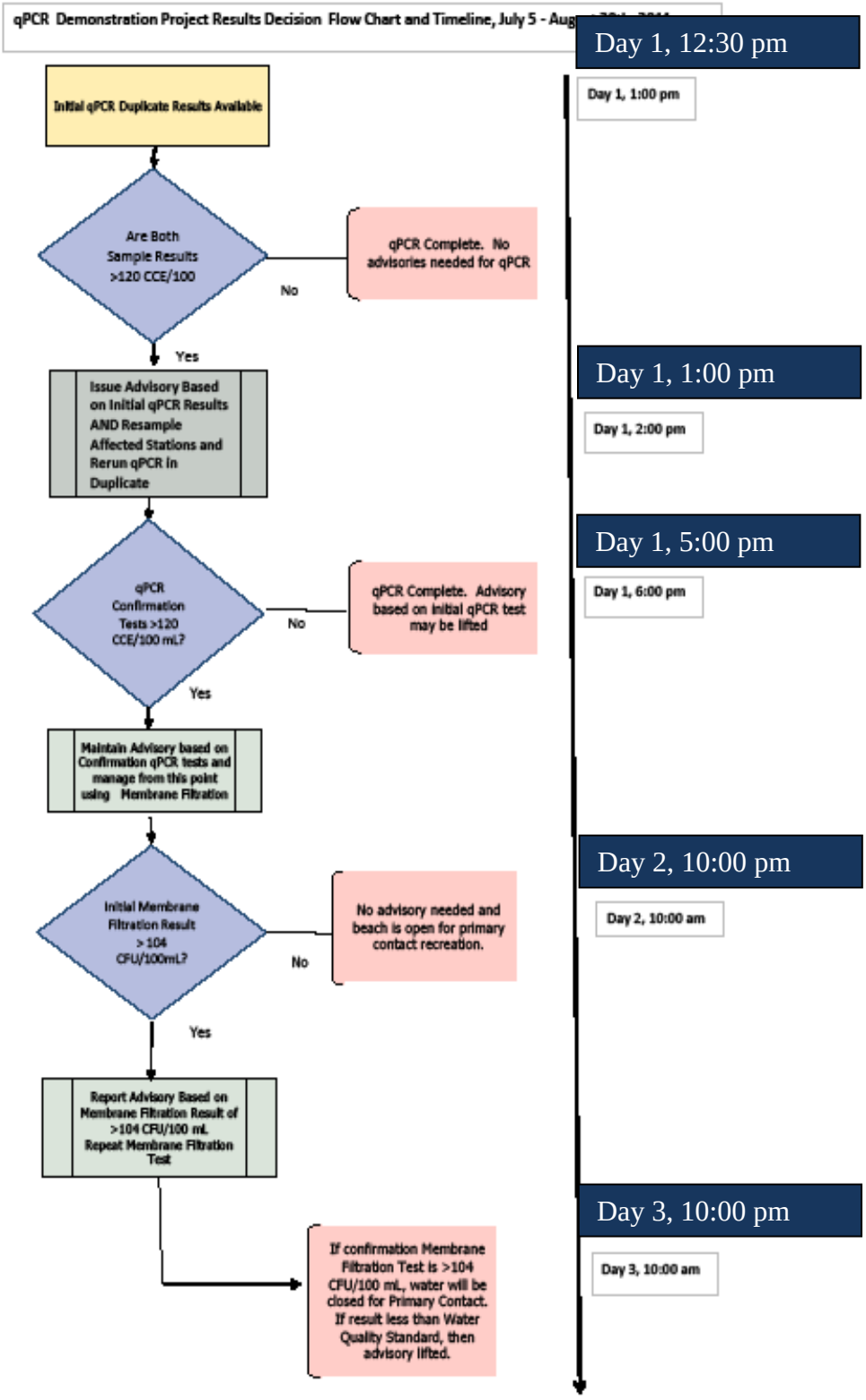

qPCR Demonstration Project Results Decision Flow Chart and Timeline, July 5 - Aug
Initial qPCR Duplicate Results Available
Are Both Sample Results >120 CCE/100
No
qPCR Complete. No advisories needed for qPCR
Yes
Issue Advisory Based on Initial qPCR Results AND Resample Affected Stations and Rerun qPCR in Duplicate
qPCR Confirmation Tests >120 CCE/100 mL?
No
qPCR Complete. Advisory based on initial qPCR test may be lifted
Yes
Maintain Advisory based on Confirmation qPCR tests and manage from this point using Membrane Filtration
Initial Membrane Filtration Result > 104 CFU/100mL?
No
No advisory needed and beach is open for primary contact recreation.
Yes
Report Advisory Based on Membrane Filtration Result of >104 CFU/100 mL Repeat Membrane Filtration Test
If confirmation Membrane Filtration Test is >104 CFU/100 mL, water will be closed for Primary Contact. If result less than Water Quality Standard, then advisory lifted.
Day 1, 12:30 pm
Day 1, 1:00 pm
Day 1, 1:00 pm
Day 1, 2:00 pm
Day 1, 5:00 pm
Day 1, 6:00 pm
Day 2, 10:00 pm
Day 2, 10:00 am
Day 3, 10:00 pm
Day 3, 10:00 am

Figure 3.

Table 5. 2011 qPCR Demonstration Project Data, Ocean County New Jersey

Date	Municipality	Station Name	Station Number	qPCR Result AM (CCE/100 mL)	qPCR PM Confirmation (if necessary)	Method 1600 Result AM (CFU/100 mL)	Current Status Code*
7/5/2011	Brick Township	Windward Beach	CCMPOC0103				
7/5/2011	Pine Beach	Avon Road	CCMPOC0118				
7/5/2011	Beachwood	Beachwood Beach	CCMPOC0116				
7/5/2011	Ocean Gate	Anglesea	CCMPOC0140				

HOW TO READ THIS TABLE

1. qPCR Samples will be analyzed in duplicate and results available by 12:30 pm
2. The maximum water quality threshold for Enterococcus using qPCR is 120 CCE/100 mL
3. If both results < than this threshold, testing using qPCR for the week is completed and results confirmed with conventional Membrane Filtration Results the Next Morning
4. If qPCR Results are > 120 CCE/100 mL and Quality Control results are acceptable, an Advisory for primary contact is issued.
5. A confirmation sample for the rapid method qPCR will be collected around 12:00 pm the same day and results will be posted by 9:00 am the following morning.
6. Membrane Filtration (traditional testing) results will then be used for ultimate determination of beach water quality and closures/postings/or advisories.
7. For more information on this new rapid method please visit www.njbeaches.org

Status Codes: 2011

O - OPEN

The most recent bacteria data indicate that levels are not above regulated water quality standards based on traditional testing or experimental rapid microbiological techniques. The Beach is open for use for primary water contact

HA - HEALTH ADVISORY

The USEPA and NJDEP are using new technology to rapidly measure levels of bacteria at this bathing beach and have detected the possible presence of bacteria at levels that may prove harmful to human health. The Ocean County Health Department and the municipality have voluntarily agreed to issue a primary contact (swimming) advisory based on the results of this new rapid test method. An advisory is NOT a closure of the beach or its facilities; rather it is an alert to the public about possible unhealthy water conditions. The advisory will be lifted if water sampled using traditional methods indicates that water quality meets the recreational bathing standard. However, the beach will be closed to swimming if traditional methods confirm that bacteria levels exceed the bathing standard. This demonstration project is being conducted to protect the public and to issue advisories in a timely manner.

C - CLOSED

Please visit www.njbeaches.org or postings and notifications from your local municipality for all beach closings or other beach closing information.

21. References

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ATTACHMENT A : Contact Information for qPCR Demonstration Project

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Municipalities

Beachwood Beach
Elizabeth Mastropasqua (Municipal Clerk)
1600 Pinewald Rd., Beachwood, NJ 08722; http://www.beachwoodusa.com/

Windward Beach
Stephen Acropolis (Mayor), Scott Pezarras, (Administrator), David Francese (Rec. Director), 401 Chambers Bridge Rd., Brick, NJ 08723; http://www.twp.brick.nj.us/

Anglesea
Paul Kennedy (Mayor and Administrator)
801 Ocean Gate Ave, CN 100, Ocean Gate, NJ 08740; http://www.boroughofoceangate-nj.gov/

Avon Road Beach
Christopher Boyle (Mayor), Charlene Carney (Municipal Clerk)
599 Pennsylvania Ave, PO Box 425, Pine Beach, NJ 08741; http://www.pinebeachborough.us/