Comparison of *Enterococcus* Measurements in Marine Beach and Bay Samples by Quantitative (real-time) Polymerase Chain Reaction, Membrane Filtration and Enterolert

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1.0 PROJECT SUMMARY

Cell densities of the fecal pollution indicator genus, *Enterococcus*, were determined by a rapid (4 h or less) quantitative polymerase chain reaction (qPCR) analysis method in 100 mL water samples collected from recreational and environmental beaches and bays in New Jersey during the summer of 2007. Measurements by this method were compared with counts of *Enterococcus* colony-forming units (CFU) determined by Method 1600, membrane filter (MF) analysis using mEI agar and Most Probable Number per 100 mLs (MPN/100 mL) using the defined substrate technology test Enterolert ® (IDEXX, Westbrook, ME). At all beaches, the geometric means of ambient *Enterococcus* concentrations in water samples (collected every two weeks at each sampling station for a total of five events for each County), exhibited lognormal distributions over the study period among all methods evaluated. The geometric means ranged from 6.8 to 188 calibrator cell equivalents (CCE) by qPCR analysis and 5.2 to 64.9 CFU by MF analysis in Monmouth County Beach/Bay Samples (*N*=204). The geometric means from the samples collected in Ocean County were 6.6 to 1785 cells by qPCR and 6.2 to 150 MPN/100 mL by Enterolert (*N*=200).

The sampling sites consisted of bathing beaches, bays, and environmental (non-swimming) areas to target samples which would potentially exhibit a wide range of *Enterococcus* concentrations. In general, when *Enterococcus* concentrations were low using MF and Enterolert ®, qPCR results followed the same trend. qPCR concentrations increased exponentially as MF or Enterolert ® results increased. This trend is due in part to the ability of qPCR to detect non-culturable or dead enterococcus. Up to a 12 fold higher amount of *Enterococcus* was detected by qPCR. However, the qPCR concentrations generally increased or decreased along with 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 (*r*) of 0.79.

Sampling was conducted between 6:00 am and 9:00 am during the study. There was one sampling event in each County in the afternoon to characterize temporal differences in

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Enterococcus concentrations among the methods. Also, sampling across a transect was performed at one of the sampling stations in Ocean County. There were differences in *Enterococcus* concentrations along the transect, but the differences were not statistically significant. There was no defined trend in *Enterococcus* concentrations in the morning versus the afternoon sampling events.

Before qPCR can be a stand alone technology for beach management decisions, additional data regarding intra- and interlaboratory variability, especially use of different qPCR platforms and reagents, must be evaluated. The qPCR protocols are more complicated as compared to the traditional Membrane Filtration techniques, and a higher level of expertise is needed to collect and evaluate the data. Other site-specific variables such as precipitation, tide height, cloud cover, salinity, temporal and spatial variability need to be further evaluated using qPCR technology at marine recreational areas. Additional side by side data collections using both qPCR and the more conventional microbiological procedures are recommended to further define and explain variability among the methods. Also, there is a need to evaluate epidemiological data in conjunction with qPCR data to help formulate appropriate risk values. Epidemiological studies are being performed by USEPA as part of the NEEAR program (National Epidemiological and Environmental Assessment of Recreation Water) study using qPCR data and Method 1600 MF procedures. The objective of the NEEAR program is to evaluate the water quality at one or two beaches per year and use these data to establish a set of risk values for gastrointestinal conditions for human health.

2.0 INTRODUCTION

There is a need for more rapid methods for the determination of microbial water quality at bathing beaches. It has been demonstrated that densities of the bacteria of the genus *Enterococcus* in both marine and freshwater samples are directly correlated with gastroenteritis illness rates in exposed swimmers (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 for 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 (MF), and Most Probable Number (Multiple Tube Fermentation (MTF) techniques and Defined Substrate Technology (DST®) tests). Although these methods have been refined over the years, results are not available for at least 18 hours. Due to the fluctuating nature of microbial contamination, this delay makes it difficult for beach managers to make decisions regarding beach closures and/or swimming restrictions. At best, decisions are made using one day old information; or a decision regarding safe beach usage is not made until results of a confirmation test are available, which may be up to 72 hours after the initial "failed" test. Because microbial water quality can change rapidly (Boehm et. al., 2002), guidelines based on indicator organisms that require 18-24 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).

The use of qPCR assays has shown promise as an alternative technology for monitoring microbial water quality at recreational beaches (Haugland, et. al. 2004; Wade, et. al, 2005). Primer sets and probes are commercially available for the specific detection of *Enterococcus* spp. using real time or quantitative PCR (qPCR) (Ludwig and Schleifer (2000), Lyon (2001), Brinkman et al. (2003), Foulds et al. (2002), Blackstone et al. (2003), Frahm and Obst (2003), Guy et al. (2003), Noble et al. (2003)). Protocols for qPCR are now available for quantifying indicator bacteria in recreational waters in less than 4 hours. Because these methods provide a more rapid assessment of water quality, they have the potential to improve decision making for those responsible for beach management decisions.

A positive correlation was observed between *Enterococcus* qPCR and the MF results at two freshwater beaches in a 10 week study conducted by Haugland et al., 2005. Although additional studies linking qPCR measurements to health outcomes or linking GI illnesses to any proposed qPCR threshold is a future need, a side by side comparison of qPCR, MF and DST® values as described in this report, is a critical step for evaluating the applicability of qPCR as a tool to manage marine bathing beaches.

One perceived drawback to qPCR is the overestimation of viable bacteria. PCR will detect and amplify dead or non culturable target organisms. If the variability and magnitude of the change of PCR values relative to the existing MF or DST® values is predictable, it may be possible to develop criteria used for beach closure decisions using PCR criteria. Beach management decisions could then be made using more timely data in a manner that limits risk to bathing beach users or prevents unnecessary closure of a public bathing beach.

The purpose of this study is to compare *Enterococcus* measurements using qPCR technology to the MF and DST® (Enterolert) testing methods using marine beach and bay waters in an attempt to evaluate a more rapid assessment protocol for recreational water quality. The sensitivity, accuracy and precision of qPCR relative to established methods are examined, as well as the ability of qPCR to measure ambient concentrations of *Enterococcus* at varying levels in the marine environment. qPCR results are compared with corresponding *Enterococcus* CFU and/or MPN counts obtained by USEPA Method 1600 and Enterolert® tests. Correlations among the three methods are performed as an initial assessment of the utility of qPCR for use in marine beach and bay *Enterococcus* analysis. Within and between sample and within station variability are assessed and compared for qPCR, MF USEPA Method 1600 and the Enterolert techniques. Also, temporal and spatial variability are evaluated from results over the course of the six week study.

3.0 METHODS AND MATERIALS
3.1 Study Sites
Twenty beach or bay locations in Ocean and Monmouth Counties, New Jersey were sampled once every two weeks between 6/18/07 and 8/20/07 (Figure 1) for this study.

Sampling Locations

Monmouth County
1 Broad St., Keyport
2 Army Recreation Beach, Sandy Hook
3 Surf Beach, Sandy Hook
4 Recreation Center, Highlands
5 Cedar Ave., Allenhurst
6 Village Beach Club, Loch Arbour
7 Myron/Wilson Bay, Neptune City
8 Newark Ave., Spring Lake
9 Brown Ave., Spring Lake
10 The Terrace, Sea Girt

Ocean County
11 Broadway, Pt. Pleasant Beach
12 Windward Beach, Brick
13 Central Ave., Island Heights
14 Sheridan Ave., Seaside Heights
15 Money Island, Toms River
16 Avon Rd., Pine Beach
17 Beachwood Beach West, Beachwood Borough
18 Anglesea Ave., Ocean Gate
19 Ocean Area 1, Island Beach
20 Bay Beach, Barnegat

Edison Perth Amboy South River Keyport Matawan Highlands Navesink Rumson Red Bank Shrewsbury Tinton Falls Asbury Park Farmingdale Point Pleasant Toms River Beachwood Seaside Park Forked River Barnegat Light Manahawkin Freehold ewood

Sussex Passaic Bergen Warren Morris Essex Hunterdon Union Hudson Somerset Middlesex Mercer Monmouth Ocean Burlington Camden Gloucester Salem Atlantic Cumberland Cape May Study Area

Miles 0 5 10 N

Figure 1. Map of Beach and Bay Sampling Stations Sampled Between June 18 – August 20, 2007, Monmouth and Ocean Counties, New Jersey

Sampling sites were selected based on historical microbiological monitoring data. A major objective of the experimental design was to establish sampling areas with a gradient of microbial densities to fully compare the sensitivities of the methods. Historical data from the Ocean and Monmouth County beach monitoring program were used to select the sampling stations (NJDEC CCMP Summary Report, 2005, 2006). The study sites were comprised of open ocean bathing beaches, bay areas, or environmental areas with documented levels of known microbial contamination. Environmental areas were included because these areas were characterized as having higher *Enterococcus* concentrations than the public bathing beach areas.

3.2 Water Sampling

Samples were collected following procedures outlined in NJDEP, Cooperative Coastal Monitoring Program (CCMP), Quality Assurance Project Plan, FY07/FY08, and 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 were collected in sterile HDPE containers in an area with a stabilized water depth between the sampler's lower thighs and chest. The sample container (250 or 500 mL sterilized HDPE wide mouth jars, Nalgene or equivalent) was placed approximately 8-12 inches below the water surface with the lid and stopper still attached. With the collector's arms extended to the front, the container was held near its base and downward at a 45-degree angle. The cap was removed and the container filled in one slow sweeping motion. The mouth of the container was kept ahead of the collector's hand and the container recapped while it is was still submerged. The cap remained submerged during sample collection. Separate bottles were used to collect samples for MF or Enterolert and qPCR samples. Sample remaining from sample filtration was used for turbidity and salinity analyses. A total of four independent (true) replicate samples were collected at each station for MF, Enterolert ®, and qPCR water samples. Conventional parameters (MF or Enterolert®) and qPCR analyses were analyzed from each replicate bottle.

Time and date of sample collection, tidal conditions, air and water temperature, rainfall, wind direction, and other general conditions were documented and recorded. Following

collection, all samples were placed in coolers with ice during transport to the laboratory and stored at 1–5 °C prior to filtration in the laboratory. Sample filtration was performed, and MF and Enterolert® tests were initiated within 6 h of collection. The filters for the qPCR analysis were frozen immediately at -20 to -70°C until analysis. The turbidity and pH of each water sample were determined by standard methods, American Public Health Association (20th Edition). Salinity was measured via conductance bridge (YSI, Model 85) or refractometer.

All samples were collected in the morning (between 6:00 and 9:00 am). There were additional samples collected in the afternoon at three stations in Ocean County on 7/30/08 and one stations in Monmouth County on 8/6/08. All samples were collected from a single point from each location with the exception of one sampling event across a transect in Monmouth County established at the Myron Wilson Bay site. At this transect, two sampling stations approximately 40 meters away on each side of the existing sampling station were established. Samples were collected at this transect on 8/6/07 at approximately 8:00 am, and repeated at 2:00 pm.

3.3 Microbiological Procedures

3.3.1 Method 1600, Membrane Filtration (MF)

Enterococcus was enumerated by EPA Method 1600 on mEI agar plates, US EPA (2002). Volumes of 10 mL from each water sample were filtered on 47-mm diameter, 0.45 μm pore size, membrane filters (Millipore Corp., Billerica, MA). The filters were incubated on plates of mEI agar for 24 hours at 41±0.5 °C before determining colony numbers. *Enterococcus* by MF was expressed as CFUs per 100 mL of water. Monthly verification tests of 10 typical and 10 atypical were performed, for each batch of water samples collected over the six week study period. Each preparation of mEI agar was tested for performance (i.e., correct enzyme reaction) using pure cultures of target and non-target organisms. Sterility of the filters and phosphate-buffered water used for rinsing the filtration apparatus was also tested with each batch of samples arriving together at the laboratory.

BioBalls™, TCS Biosciences, LTD, which contain a certified number of bacterium, were used routinely for determination of Ongoing Precision Recovery.

3.3.2 Enterolert ® Method, IDEXX

Enterolert ® provides an MPN result based on the presence or absence of fluorescence in 101 individual wells. Each well contains a sample-nutrient indicator mixture. Enterolert analysis on all samples collected in Ocean County was performed by the Ocean County Utilities Authority (OCUA), Bayville, New Jersey. A 1:10 dilution of the test water sample was prepared (90 mLs of sterile Buffer plus 10 mL of sample) in a sterile container. A package of powdered Enterolert regent was then added to the container and the sample solution was mixed and poured into a Quantitray, a sterile plastic disposable panel containing 51 wells. The tray was then mechanically sealed after distribution of the mixture into the wells and incubated for 24 h at $41.0 \pm 5^{\circ}C$. Enterolert ® uses a nutrient-indicator to detect *Enterococcus*. This nutrient-indicator fluoresces when metabolized by *Enterococcus*. Test results were read in a darkened room. The tray was placed under a 365-nm-wavelenth UV light with a 6 W bulb as supplied by IDEXX, Westbrook, Maine, and the number of positive wells was enumerated. Any fluorescence in a well was considered a positive reaction for that well. MPN tables were used, and the number of positive wells with a 10X dilution factor, were used to determine the density of *Enterococcus* per 100mL of sample. Ongoing Precision and Recovery samples were performed on a monthly basis in addition to the required media viability checks. The sterility of the sample containers used was checked by OCUA prior to first use. Sterile water used for the Enterolert® testing was evaluated once per lot prior to first use.

Enterolert ® is an EPA approved method for wastewaters and ambient waters. A strong positive correlation with Membrane Filtration has been established for both fresh and marine water samples using this method (Budnick, 1996). MF and Enterolert ® results from Monmouth and Ocean County samples were treated similarly and compared directly to the qPCR results.

3.4 qPCR Procedures

DNA extraction, amplification and detection of *Enterococcus* were based on US EPA Draft Method 1606, Brinkman (2002) and Haugland (2005).

3.4.1 Test Sample Filtration Procedure

Fifty mLs of each test sample were filtered through a 0.4 micron, 47 mm diameter polycarbonate filter fitted in a pre-sterilized disposable 250 mL filter funnel within 6 hours of collection. The filter paper was folded in half and folded longitudinally 2-3 more times before being placed into a 2.0 mL polycarbonate preloaded bead tube (Gene-Rite S0201-50) using sterile forceps. The tubes with the polycarbonate filter paper were frozen at -20 to -70 until ready for use in the qPCR analysis.

3.4.2 Test Filter Sample Extraction Procedure

Salmon testes DNA extraction buffer was prepared in advance of the DNA extraction procedure. Salmon testes DNA extraction buffer acts as an exogenous, internal positive control and reference. Initially, the concentrated salmon testes DNA (Sigma, D1626, and St. Louis, MO) was resuspended in water and was diluted with AE buffer (Qiagen, Cat No. 19077, Valencia, CA) to obtain the target concentration required for the procedure. 590 uL of a 0.2 ug/mL of salmon testes DNA extraction buffer mix was added to 2.0 mL tubes containing silica beads (GeneRite, #S0205-50, North Brunswick, NJ) and the negative control filter blank or test sample filter. The extraction tubes were subjected to bead beating in an eight position mini bead beater (Biospec Corp., Bartlesville, OK) for 1 minute at a rate of 5,000 rpm and were then centrifuged at 12,000 x g for 1 minute to pellet the glass beads and debris. The genomic DNA in the supernatants from the extraction tubes was transferred to sterile 1.7 mL microcentrifuge tubes and then centrifuged for additional 5 minutes at 12,000 x g to further purify the DNA. The final genomic supernatant was either analyzed immediately or stored at -20 °C until analysis by qPCR.

3.4.3 Enterococcus faecalis culture procedure

A pure culture of *E. faecalis*, ATCC 29212, was inoculated in a 20 mL test tube with 10 mL brain heart infusion broth (BHI, Difco, #Ref 237500, Sparks, MD) and was incubated on a shaker for 24 ± 2 hours at 35 °C $\pm$ 0.5 °C. Also, an uninoculated tube was placed in the incubator to test the sterility of BHI broth. The cell culture was transferred to 15 mL conical tubes and centrifuged at 6000 x g for 5 minutes to pellet the cells. The supernatant was discarded, and the cell pellet was washed twice with 10 mL of a 1x phosphate-buffered saline (PBS) (Invitrogen, Cat. No. 14190, Carlsbad, CA) and resuspended in 5 mL of 1x PBS solution. The *E. faecalis* cell suspension was quantified on the NanoDrop spectrophotometer ND-1000 v3.3.1 (Wilmington, DE). The *E. faecalis* cell suspension was divided into 6 microcentrifuge tubes, each one containing 500 uL for a standard curve determination. The remaining cell suspension was dispensed by 10 uL aliquots into 100-200 microcentrifuge tubes, which acted as calibrator samples for subsequent qPCR analyses.

3.4.4 Calibrator and DNA extraction and preparation procedure

A 10 uL aliquot of *E. faecalis* cell suspensions was spotted onto a blank polycarbonate filter which contained 590 uL of 0.2 ug/mL Salmon testes DNA extraction buffer in the pre-loaded glass beads extraction tube as described in section 3.4.2. The tube was shaken by a mini-bead beater (Biospec Corp., Bartlesville, OK) for 1 minute at 5,000 rpm and then centrifuged at 12,000 x g for 1 minute to pellet the glass beads and debris. The genomic DNA in the supernatants from the extraction tubes was transferred to sterile 1.7 mL microcentrifuge tubes and then centrifuged for additional 5 minutes at 12,000 x g to purify the DNA. The final genomic supernatant was either analyzed immediately or stored at -20 °C until analysis.

The *E. faecalis* cell suspensions used to prepare the calibrator samples were also used to create a standard curve for *E. faecalis*. Two 500 uL undiluted *E. faecalis*

cell suspensions were placed into a 2.0 mL preloaded tube containing glass beads, placed in the mini-bead beater, and centrifuged. The supernatant was transferred to another tube. The genomic DNA supernatant was then digested with 1 uL of 5 ug/uL RNase A (Sigma, R-4642, St. Louis, MO) for 1 hour at 35 °C. The RNase A was used to digest the RNA in the sample to facilitate purification of the genomic DNA of *E. faecalis*. After RNase digestion, the DNA was purified by DNA-EZ purification kit (GeneRite, K102-02C-50, North Brunswick, NJ). The concentration of DNA was then measured on the NanoDrop spectrophotometer. The DNA was considered to be acceptable if the OD_{260}/OD_{280} reading was ≥ 1.75.

3.4.5 qPCR assay preparation and detection procedure

Each reaction tube contained assay mix with a total volume of 25 uL. The assay was designed to target the large subunit ribosomal RNA (lsrRNA) gene sequences from all known species of *Enterococcus* bacteria in water (USEPA 2006, Draft Method 1606). The qPCR assay mix had the following components: 12.5 uL of TaqMan Universal Master Mix (Applied Biosystems, Part Number 4304437), 2x concentrated; 1 uL of forward primer (1 μM); 1 uL reverse primer (1 μM); 1.5 uL of a fluorogenic probe (0.08 μM); 2.5 uL of 2mg/mL ultra pure bovine serum albumin (Ambion, Cat # AM2616); 1.5 uL of sterile water and 5 uL of diluted DNA template (5 fold dilution). TaqMan Universal Master Mix consisted of AmpliTaqGold DNA polymerase, AmpErase UNG, dNTPs with UTP, passive reference 1 and optimized buffer components. The published primer and probe sequences were ECST748F: 50-AGAAATTCCAAACGAACTTG, ENC854R: 50-CAGTGCTCTACCTCCATCATT and GPL813TQ: 50-6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA for the probe (Ludwig and Schleifer, 2000). These sequences are homologous to the large subunit ribosomal RNA genes of all reported species within the *Enterococcus* genera. Published primers and hybridization probe sequences for salmon DNA assay were SketaF2: 50-GGTTTCCGCAGCTGGG for the forward primer; SketaR3: 50-CCGAGCCGTCCTGGTCTA for the reverse primer; and SketaP2: 50-6FAM-AGTCGCAGGCGGCCACCGT- TAMRA for the probe. These sequences are

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homologous to internal transcribed spacer region 2 of the ribosomal RNA gene operon of chum salmon, Oncorhynchus keta, Domanico et al. (1997). Primers and fluorescently labeled probes were purchased from Applied Biosystems Inc. (Foster City, CA).

Each reaction tube containing 25 uL of the assay mix was then place in the Smart Cycler II for 45 cycles under the following conditions: 2 min at 50 °C, 10 min at 95 °C, 15 s at 95 °C and 2 min at 60 °C. Cycle threshold (C_T) values were calculated by the instrument. Cycle threshold values occurred when the point of the amplification curve crossed a fluorescence threshold that was established for this qPCR method. C_T values for each sample were collected at the end of each run and saved in Excel format. A No Template Control (NTC), which tests the assay mix for contamination, was performed with each batch of samples analyzed along with one field blank. Any positive amplification for no-template control and filter blank samples were reanalyzed for verification. A sample was considered below the limit of detection when there was no threshold after 45 cycles (USEPA, 2006).

3.5 qPCR Quality Control

Maintaining a contamination free process and environment is an important component of qPCR analysis. Decontamination of workstations, pipettes and equipment after each use was performed using a 70% ethanol solution. Presterilized pipets manufactured with aerosol resistant filters were used in the steps of the DNA testing process. Pre-sterilized disposable filters and housings were used during the test sample filtration process to prevent cross-over contamination. All reagents and supplies were tested and certified by the manufacture for specificity, sensitivity and to be free of contamination. The primers and probes were dispensed in small aliquots to avoid contamination and degradation.

Contamination or misleading qPCR results can be detected by using positive and negative quality control samples, which were implemented throughout this study. A negative control known, as a method blank, was performed in the lab after sample collection to

test for proper filtering technique and reagent sterility. A method blank was performed by using DNase/RNase free sterile water as a sample, which was processed in parallel with the water samples. Another negative control was a No Template Control (NTC) which was used in each batch of samples tested by qPCR to verify the purity of the master mix, reagents, and ensure no contamination occurred during the processing of the test samples. A NTC consisted of DNase/RNase free sterile water or buffer.

A positive control called a Sample Processing Control (SPC) was used to validate that the master mix and reagents were prepared properly by producing amplification of the target nucleic acid. A positive control that does not produce amplification of DNA during a qPCR run would indicate that there were interferences from sample contamination or a problem with the reagents.

3.6 qPCR Data Analysis

Amplification efficiency of the standard curve was the first step in the qPCR data analysis process. Amplification efficiency is defined as the rate at which a PCR amplicon was generated, doubling the number of copies during each cycle (Applied Biosystem, 2004). The amplification efficiency is normally equal to 2. However, the reagents, assay preparation, purity of the samples and the data analysis can alter the efficiency to less than 2. Initially, DNA from *E. faecalis* suspended cell stocks were serial diluted with the following concentrations 4×10^4, 4×10^3, 4×10^2, 2×10^2, and 1×10^2 lsrRNA gene sequences per 5 uL. Each was analyzed by qPCR in triplicate. C_T values were obtained, averaged and subjected to regression analysis against the log 10-transformed target sequence per reaction in order to obtain the equation of the line for the standard curve. The DNA standard curve for this study was $y = -3.44x + 35.792$, where -3.44 is the slope. The slope value from the standard curve was used to calculate the amplification efficiency using the following formula $AF = 10 \wedge (1/(-)\text{slope value})$. The calculated AF was 1.954. The r^2 value from the DNA standard curve used during this study was 0.999

This amplification efficiency value was used in conjunction with the comparative cycle threshold method to quantify the target cells in the water filtrate extracts (Applied Biosystem (2004), Haugland et al (1999)). Target cells were expressed as calibrator cell equivalents (CCE). Initially, the quantity of target organisms (Δ C_T) in a calibrator sample were determined by subtracting Salmon DNA C_T value from *Enterococcus* C_T value. The average of duplicate calibrator samples were used to calculate a Δ C_T value. The Δ C_T value from the calibrator samples was used to compare against the Δ C_T from test samples to calculate calibrator cell equivalents (CCE). CCE was the endpoint used for all qPCR analyses.

Similar to calibrator samples, Δ C_T for test samples were calculated by subtracting water samples C_T from salmon DNA C_T. $\Delta\Delta C_T$ was obtained by subtracting ΔC_T for test samples from ΔC_T for calibrator samples. The ratios of target sequences in the test sample and calibrator samples were calculated by using the formula $(E + 1)^{-\Delta\Delta CT}$. These ratios were than multiplied by the known number of target organism cells in the calibrator samples to obtain estimates of the numbers of target cells in each test sample. Target cells in each test sample were then multiplied by 5 to account for the dilution of the test sample and calibrator extracts. A separate multiplication factor was used to express as 100 mL sample volumes.

Five-fold dilutions of the test sample filters and calibration extracts were analyzed in this study to minimize potential interferences from undiluted saltwater samples. The C_T values for salmon DNA assay in water filter samples with higher than 3 C_T units above the mean values from the calibration extracts were reanalyzed. If reanalysis did not fall within expected results, the data may be qualified as an estimated value.

3.7 Statistical Analysis

Arithmetic and geometric means were calculated on all microbiological results collected during the study. A Log_{10} transformation was performed on all raw data. Standard deviation between and within sampling visits was determined on the Log10 transformed data. Coefficient of Variation (C.V.) calculations of within sampling visits raw

geometric means were performed on data from each sampling station. A linear regression was calculated using the geometric means of MF/Enterolert versus qPCR results. Two-way unpaired t-tests were performed on samples collected in the morning and afternoon (p=0.05) to determine significant differences. Differences in *Enterococcus* concentrations across a sampling transect were tested for assumptions of Normality and Variance using Log_{10} transformed data. Significant differences of *Enterococcus* concentrations across a transect were determined using either Tukey's Method of Multiple Comparisons (p=0.05, critical value = 4.49, parametric) or Kruskal Walis/Dunns Multiple Comparison Test, (p=0.05; crit value = 2.936, non-parametric).

4.0   RESULTS AND DISCUSSION

Sampling stations were selected to provide a range of *Enterococcus* concentrations. Environmental stations, i.e., non-bathing beach stations, were included in the study design because the established bathing beach sampling areas typically do not exhibit *Enterococcus* concentrations near or above the single sample maximum water quality criterion of 104 CFU/mL. Arithmetic mean *Enterococcus* concentrations using MF/Enterolert ® ranged from 5.3 to 261 CFU or MPN/100mL in Ocean and Monmouth Counties over the entire sampling period (Table 1). Arithmetic mean CCE values for qPCR analysis ranged from 14.8 to 2745 CCE/100 mL (Table 1).

Enterococcus were detected by either MF or Enterolert ® Methods in 60.4% of the samples (Total N=404). *Enterococcus* DNA was detected in 77% of the samples via qPCR. Samples with no detectable bacterium were assigned values of 5 CFU or MPN per 100 mLs (equivalent to one-half the detection limit of the MF and Enterolert Methods), and those with no detectable CCE's were assigned 2.5 CCE for qPCR analysis, for calculating the geometric means and standard deviations of individual sample results for the study. Approximately 10% of the samples collected from Monmouth county (n=204) resulted in a CFU concentration of 104 or greater. This percentage was doubled in Ocean County. 41 out of the 200 samples analyzed for *Enterococcus* by MF (20.5%) resulted in values greater than 104 MPN/100mL.

Figures 2a-j and 3 a-j show the geometric means of *Enterococcus* densities at all sampling locations measured over the course of the study. The charts are arranged from lowest to highest geometric means for MF or Enterolert ®. In general, sampling areas with low concentrations of *Enterococcus* as measured by MF or Enterolert ®, also had low levels of *Enterococcus* via qPCR. At stations with detectable levels of *Enterococcus*, qPCR concentrations ranged between ½ and 1 order of magnitude higher than MF/Enterolert ® results (Figures 2 and 3). This is to be expected since qPCR has the ability to detect DNA from culturable as well as non-culturable or dead organisms. However, an important trend to observe is that the relative changes in MF or Enterolert ® from week to week were reflected in the qPCR values, which changed in the same direction.

A scatter plot and regression analysis of qPCR versus MF/Enterolert ® geometric mean densities of *Enterococcus* from all sampling visits is presented in Figure 4. The overall correlation coefficient (r) between the results of qPCR and MF/Enterolert ® was 0.79. This strong correlation is higher than the value previously reported by Haugland et. al. 2005 in a qPCR method comparison study of two freshwater bathing beaches.

The average geometric means at each sampling area are presented by county in Tables 2a (Monmouth County) and 2b (Ocean County). The sampling areas are arranged in order of low to high based on the MF/Enterolert ® geometric means from all analysis at each area. A major objective of the sampling plan was to select stations which would be represented by *Enterococcus* over a wide range of concentrations. qPCR geometric mean concentrations were higher than corresponding MF/Enterolert ® at all sampling areas except The Terrace, Monmouth County. This site contained relatively low levels of *Enterococcus*. Each Monmouth County sampling area was ranked from 1 to 10 based on

Table 1. Summary of Arithmetic Mean *Enterococcus* Concentrations of Ocean and Monmouth County Bathing Beach and Bay Samples using qPCR and Membrane Filtration or Enterolert®, June 18 – August 20, 2007

| Sampling Area	MF (CFU/100 mL) or Enterolert® (MPN/100 mL) n = 20	qPCR (CCE/100 mL) n=20	County	Recreational Or Environmental Station
Ocean Area 1	6.5	16.4	Ocean County	Recreational
Sheridan Ave.	8.5	39.6	Ocean County	Recreational
Bay Beach	13.5	45.1	Ocean County	Recreational
Broadway	13.3	45.6	Ocean County	Recreational
Windward Beach	86.5	239	Ocean County	Recreational
Anglesea Ave.	101	802	Ocean County	Recreational
Avon Road	124	780	Ocean County	Recreational
Money Island	135	785	Ocean County	Recreational
Beachwood Beach	145	920	Ocean County	Recreational
Central Ave.	261	2745	Ocean County	Environmental
Army Rec Center	5.3	14.8	Monmouth County	Recreational
Surf Beach	7.3	44.1	Monmouth County	Recreational
Newark Ave.	7.8	46.2	Monmouth County	Recreational
The Terrace	13.5	24.6	Monmouth County	Recreational
Village Beach Club	15.5	116	Monmouth County	Recreational
Cedar Ave.	28.0	151	Monmouth County	Recreational
Brown Ave.	42.3	78.0	Monmouth County	Recreational
Broad Street	40.0	229	Monmouth County	Environmental
Rec Center	74	365	Monmouth County	Recreational
Myron/ Wilson Bay	123	398	Monmouth County	Environmental
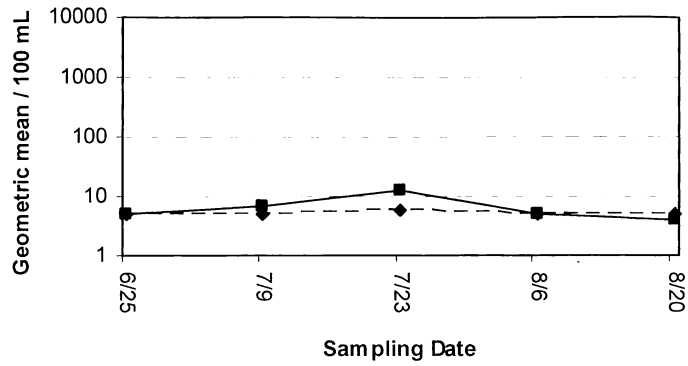
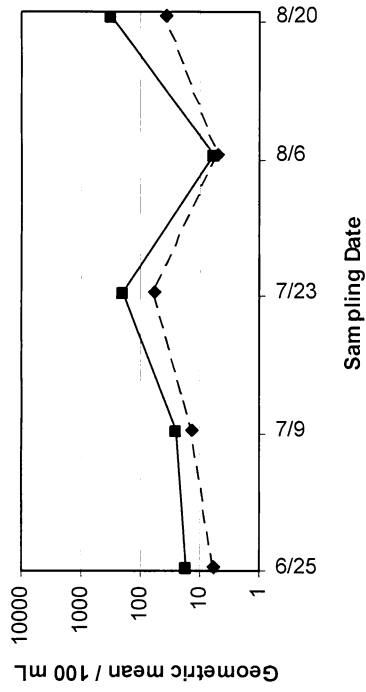a) *Army Recreational Beach*
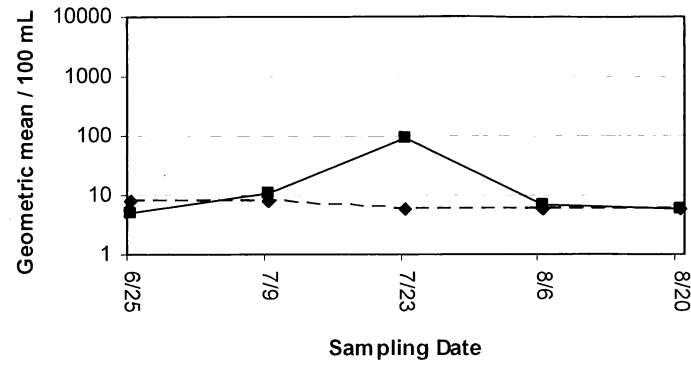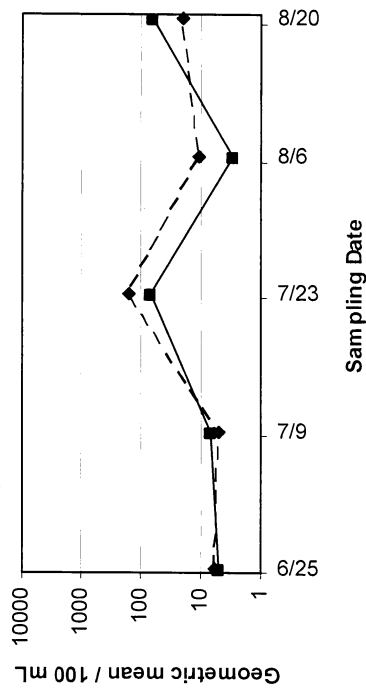

b) *Surf Beach*
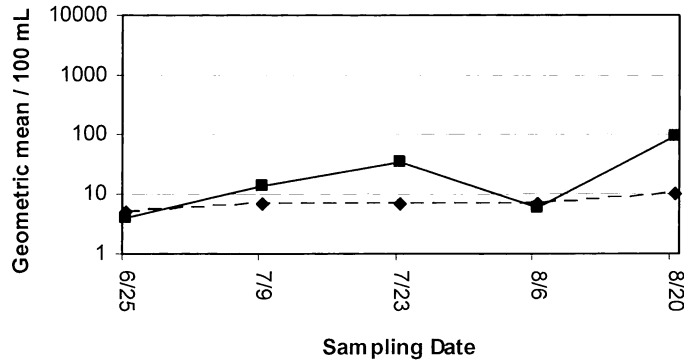
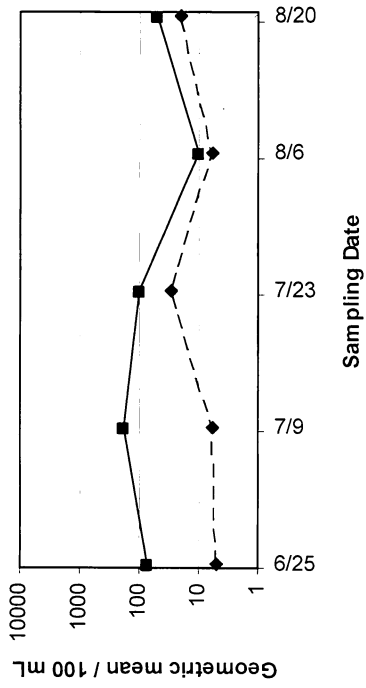c)*Newark Avenue*
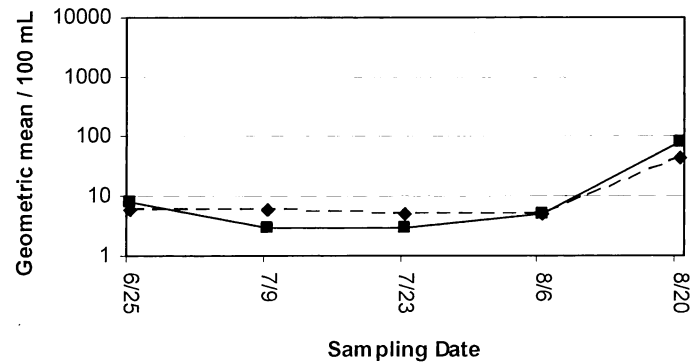
d) *The Terrace*

Figure 2a. – 2d. Geometric means of *Enterococcus* densities per 100mL of water from all sampling locations at a) Army Recreation Beach; b) Surf Beach; c) Newark Ave.; and d) The Terrace. The geometric means are calculated using four replicates for each method. qPCR Results are designated with a (■) on a solid line; and MF results are designated with a (♦) on a dotted line.

Figure 2e. – 2h. Geometric means of *Enterococcus* densities per 100mL of water from all sampling locations at e) Village Beach Club; f) Cedar Ave.; g) Broad Street; and h) Brown Avenue. The geometric means are calculated using four replicates for each method. qPCR Results are designated with a (■) on a solid line; and MF results are designated with a (♦) on a dotted line.
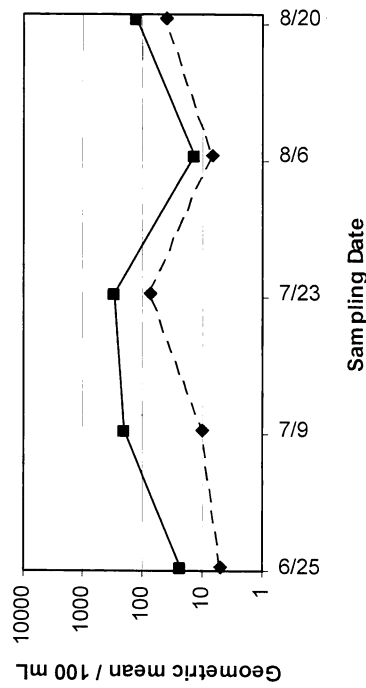
8/20

8/6

7/23

7/9

6/25

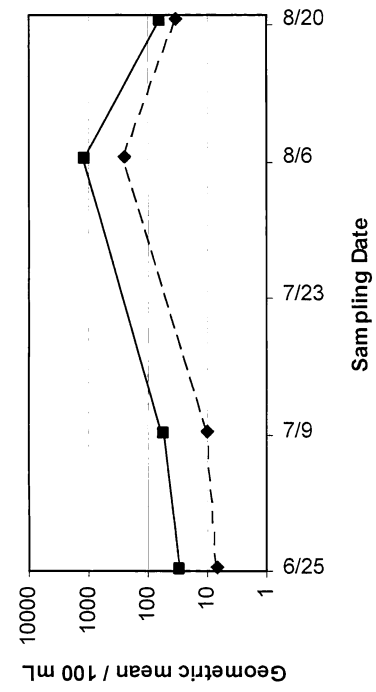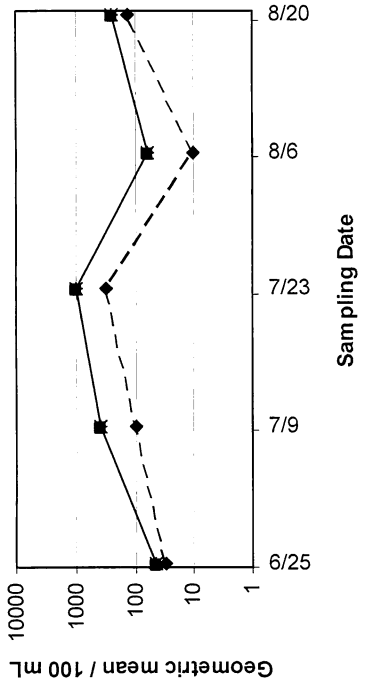Sampling Date

j) Myron/Wilson Bay

10000

1000

100

10

1

Geometric mean / 100 mL

8/20

8/6

7/23

7/9

6/25

Sampling Date

i) Recreation Center

10000

1000

100

10

1

Geometric mean / 100 mL

Figure 2i. – 2j. Geometric means of *Enterococcus* densities per 100mL of water from all sampling locations at i) Recreation Center and j) Myron/Wilson Bay. The geometric means are calculated using four replicates for each method. No results reported on 7/23 for the Recreation Center site due to non-quantified MF results. qPCR Results are designated with a (■) on a solid line, and MF results are designated with a (♦) on a dotted line.
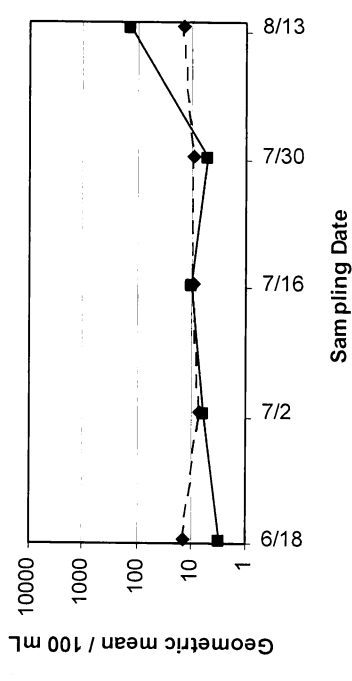
Figure 3a. – 3d. Geometric means of *Enterococcus* densities per 100mL of water from all sampling locations at a) Ocean Area 1; b) Sheridan Avenue; c) Broadway Ave.; and d) Bay Beach. The geometric means are calculated using four replicates for each method. qPCR Results are designated with a (◆) on a solid line; and Enterolert® results are designated with a (◆) on a dotted line.
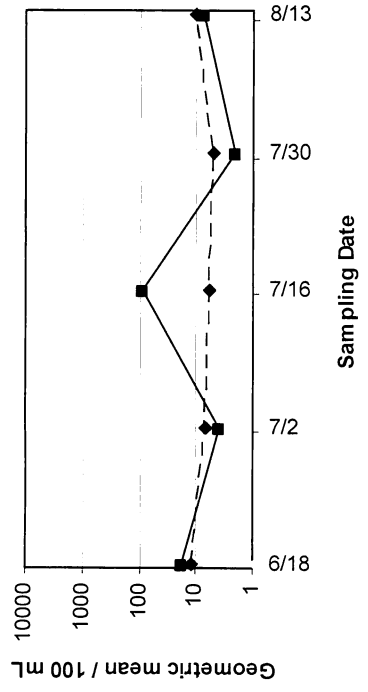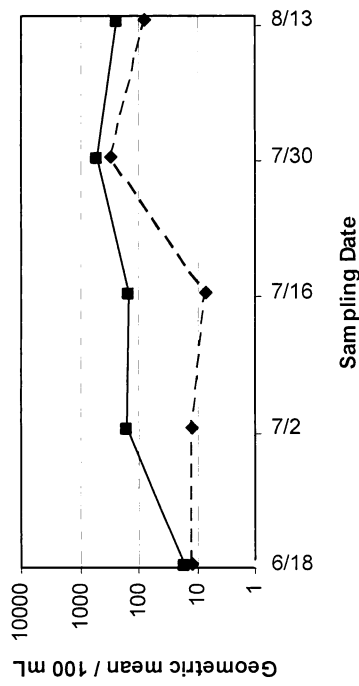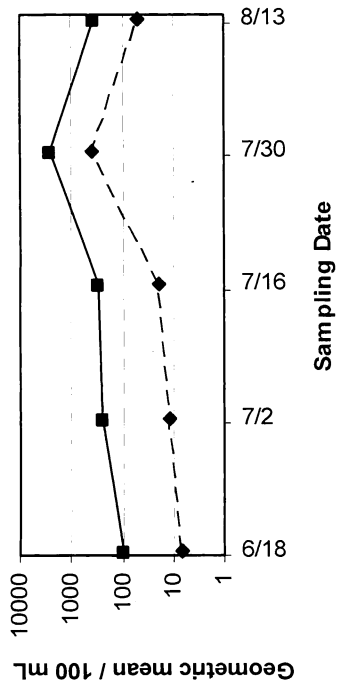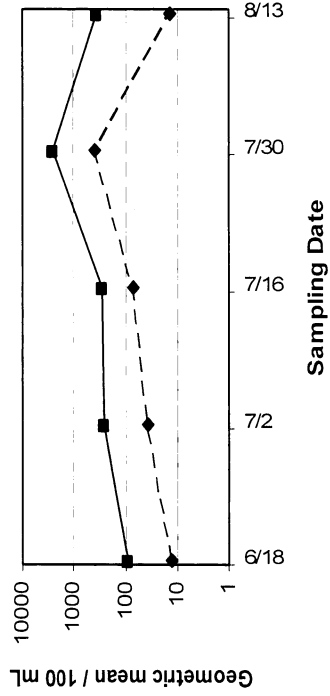
Figure 3e. – 3h. Geometric means of *Enterococcus* densities per 100mL of water from all sampling locations at e) Windward Beach; f) Anglesea Avenue; g) Avon Road West; and h) Money Island. The geometric means are calculated using four replicates for each method. qPCR Results are designated with a (■) on a solid line; and Enterolert® results are designated with a (◆) on a dotted line.
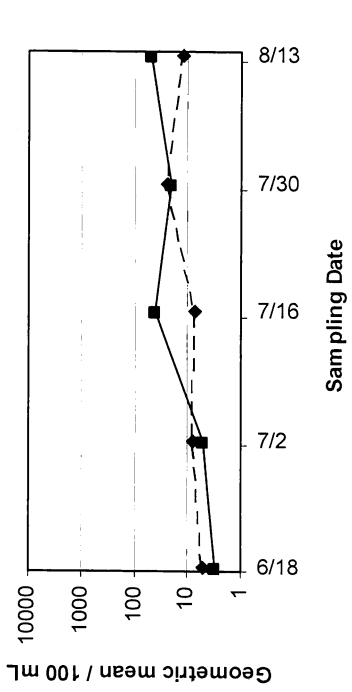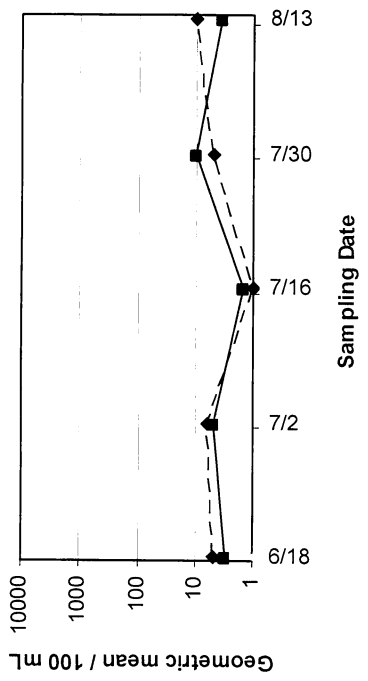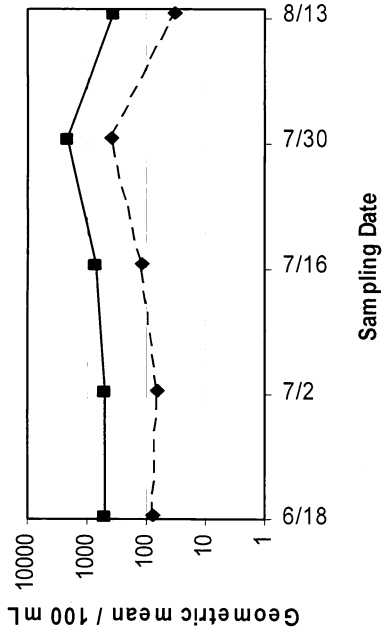
j) Central Avenue

i) Beachwood Beach
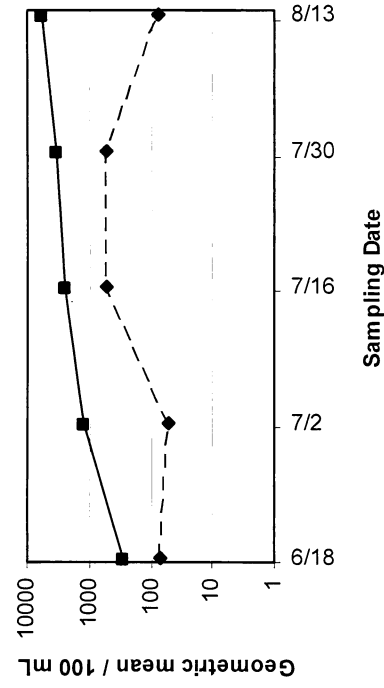
Figure 3i. – 3j. Geometric means of *Enterococcus* densities per 100mL of water from all sampling locations at i) Beachwood Beach and j) Central Avenue. The geometric means are calculated using four replicates for each method. qPCR Results are designated with a (■) on a solid line; and Enterolert® results are designated with a (◆) on a dotted line.
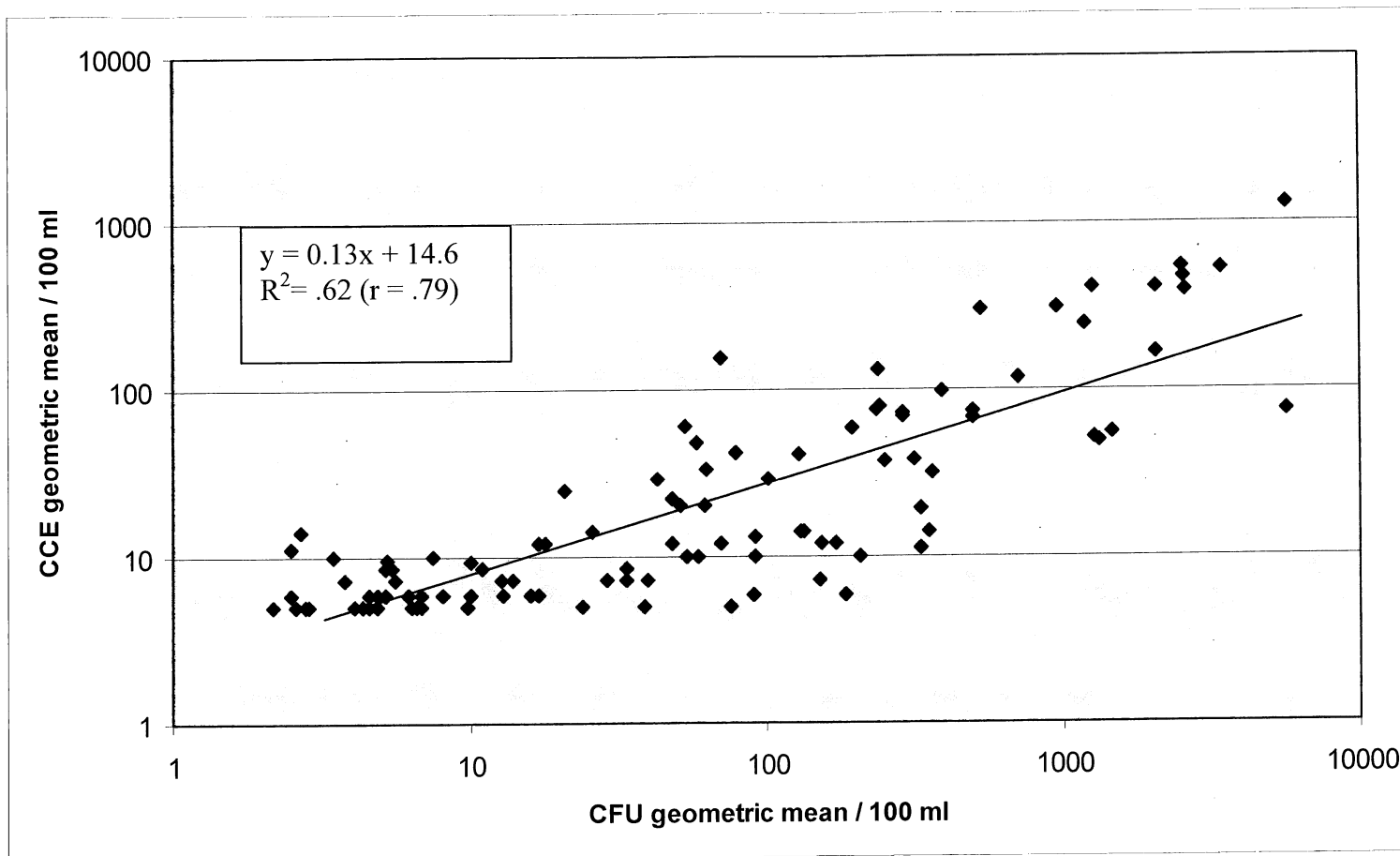
Figure 4. Scatter plot and regression analysis results of geometric mean *Enterococcus* CFU/MPN densities, Determined by MF (Method 1600) or Enterolert Methods vs. *Enterococcus* CCE, determined by qPCR Method 1606 for all sampling visits to beaches, bays, and environmental sampling areas in Ocean and Monmouth Counties, NJ, June 18 – August 20, 2007.

the *Enterococcus* geometric means using MF and qPCR (with 1 being the lowest geometric mean and 10 the highest) and those in Ocean County using Enterolert® and qPCR (Tables 2a. and 2b). qPCR relative ranks were within 1 for Enterolert® at all Ocean County stations (Table 2b) and 3 ranks within MF results in Monmouth County (Table 2a). Although qPCR concentrations were higher overall than corresponding MF or Enterolert ®, the qPCR technology was able to discern differences in contamination at levels very similar to the conventional *Enterococcus* methods.

At the sites which had relatively low levels of *Enterococcus* using MF or Enterolert ®, the corresponding CCE's were also low (Tables 2a. and 2b). There were up to five fold higher qPCR concentrations (Broad Street, Table 2a) compared to the MF geometric mean at Monmouth County sites, and up to a 12 fold higher qPCR concentration compared to the Enterolert ® geometric mean for Ocean County (Table 2b). The trend from this study indicates that qPCR *Enterococcus* concentrations are comparable to MF or Enterolert ® at sites where contamination is low or not detected. However, qPCR concentrations increase at a greater rate relative to the MF/Enterolert ® concentrations as *Enterococcus* contamination increases.

The standard deviation values shown in Tables 2a and 2b indicate that for qPCR within sampling visit variability was generally higher than for MF/Enterolert ®. This is not surprising, given the ability of qPCR to detect non-culturable or dead *Enterococcus*, and it would affect the within difference standard deviations at the more pristine sites. The differences in standard deviation among sampling visits were not as remarkable between qPCR and MF/Enterolert ® results (Tables 2a and 2b). This result may be due to the rather large number of samples with low *Enterococcus* concentrations. Over 65 percent of the stations were characterized with MF or Enterolert concentrations of 20 CFU's or MPN per 100 mL or less. In contrast to Haugland et. al. 2005, *Enterococcus* measurements by qPCR were considerably higher than MF/Enterolert ® at the stations which contained little or no *Enterococcus*.

Table 2a. Summary of *Enterococcus* beach and bay water analysis results from qPCR and MF analysis, Monmouth County, NJ, in increasing Membrane Filtration order.

	Average Geometric Mean n= 20		Rank (1 Low – 10 High)		Log10 SD AMONG Sampling Visits		Log10 SD WITHIN Sampling Visits		C.V. WITHIN Sampling Visits Raw Data	
	Membrane Filt. (CFU/100 mL)	qPCR (CCE/100mL)	Enterolert	qPCR	Enterolert	qPCR	Enterolert	qPCR	Enterolert	qPCR
Army Rec Center	5.2	6.8	1	1	.07	.49	.07	.51	.21	1.43
Surf Beach	6.8	13.0	2	3	.03	.32	.15	.69	.34	1.20
Newark Ave.	7.1	17.2	3	5	.06	.42	.17	.62	.46	.83
The Terrace	8.2	7.9	4	2	.40	.56	.12	.36	.59	.87
Village Beach Club	10.0	60.3	5	8	.33	.38	.24	.53	.79	.83
Cedar Ave.	15.7	45.8	6	6	.46	.62	.27	.63	.62	1.07
Brown Ave.	15.9	14.4	7	4	.59	.51	.26	.75	.59	1.30
Broad Street	16.0	77.2	8	7	.47	.50	.38	.57	1.00	1.42
Rec Center	27.4	106	9	9	.70	.65	.13	.58	.30	.41
Myron/ Wilson Bay	64.9	188	10	10	.57	.51	.23	.43	.48	.84
Column ID	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*

Table 2b. Summary of *Enterococcus* beach and bay water analysis results from qPCR and MF analysis, Ocean County, NJ, increasing order of Enterolert.

|	Average Geometric Mean n= 20		Rank (1 Low – 10 High)		Log10 SD BETWEEN Sampling Visits		Log10 SD WITHIN Sampling Visits		C.V. WITHIN Sampling Visits Raw Data	
	Enterolert (MPN/100 mL)	qPCR (CCE/100mL)	MF	qPCR	MF	qPCR	MF	qPCR	MF	qPCR
Ocean Area 1	6.2	6.6	1	1	.11	.10	.10	.51	.24	1.79
Sheridan Ave.	7.6	10.8	2	2	.13	.53	.16	.51	.44	1.49
Bay Beach	9.8	14.2	3	4	.23	.40	.25	.58	.85	1.36
Broadway	10.4	11.0	4	3	.09	.58	.31	.50	.83	1.19
Windward Beach	29.9	138	5	6	.68	.51	.25	.46	.46	.43
Anglesea Ave.	31.1	137	6	5	.67	.77	.23	.78	.59	1.54
Avon Road	46.7	360	7	8	.62	.49	.23	.40	.38	.57
Money Island	54.9	327	8	7	.72	.69	.19	.37	.21	.35
Beachwood Beach	94.9	664	9	9	.40	.26	.16	.27	.43	.59
Central Ave.	150.2	1785	10	10	.49	.49	.21	.23	.40	.31
Column ID	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*

This study supports the continued evaluation of qPCR as a potentially effective monitoring tool for beach management decisions. Before a reliable and defensible qPCR risk value for *Enterococcus* is developed, additional data are needed. There is a need to evaluate epidemiological data in conjunction with qPCR data to help formulate appropriate risk values. Epidemiological studies are being performed by USEPA as part of the NEEAR Program Study (National Epidemiological and Environmental Assessment of Recreation Water) study using qPCR data and the Method 1600 MF procedure. The objectives of this program are to evaluate the water quality at one or two beaches per year and ultimately obtain a new set of health and water quality data for the qPCR *Enterococcus* and *E. coli* protocols. Inter- and Inner laboratory variability, as well as effects of physical and chemical parameters as they relate to qPCR performance is needed. Protocol refinement and the performance of the many different qPCR platforms is also another identified need prior to establishment of a qPCR based human health risk value for *Enterococcus*.

4.1 Temporal Factors Affecting *Enterococcus* Concentrations

Samples collected in the morning at three locations in Ocean County (July 30, 2007, 7:00 am) and one location in Monmouth County (August 6, 2007, 07:45 am) were resampled in the afternoon (between 12:00 pm and 2:00 pm) to evaluate temporal factors related to MF, Enterolert ®, and qPCR results. At Beachwood Beach in Ocean County, both qPCR and Enterolert ® values were higher in the afternoon (Figure 5b.). A contrasting result was observed at Central Avenue in Ocean County (Figure 5a.). At Windward Beach in Ocean County, qPCR was higher in the afternoon sample, but Enterolert ® results were lower in the afternoon (Figure 5c). Overall, there was not a discernable trend with regard to *Enterococcus* densities between morning and afternoon.

Data from the EMPACT Beaches Project (USEPA 2005), using E. *coli* and *Enterococcus* by MF, found that indicator levels generally decreased by the afternoon at four of the beaches studied, and there was no trend detected at one freshwater beach which generally had low levels of *E. coli* and *Enterococcus* at all times. In contrast,

32

a) Central Avenue, Ocean County NJ
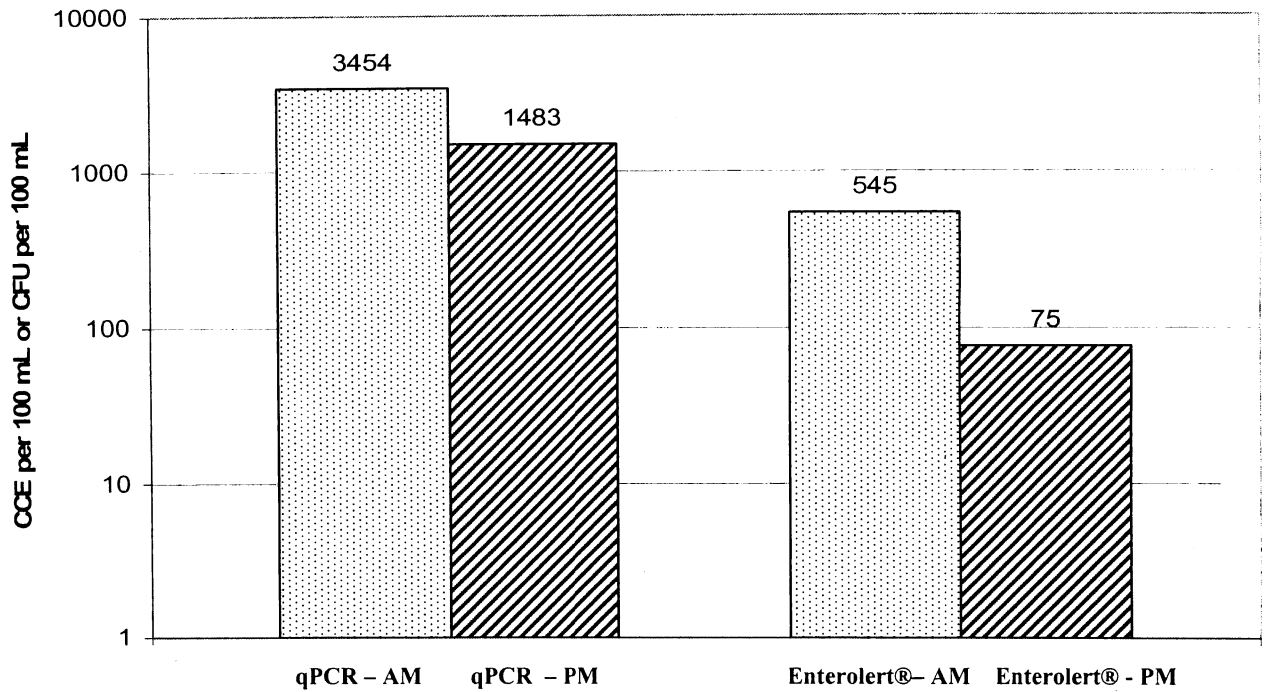
b) Beachwood Beach, Ocean County NJ

Figure 5a. & 5b. Densities as geometric means (n=4) of *Enterococcus* using qPCR Method 1606 (CCE) and Enterolert® (MPN/100 mLs) on samples collected *at Central Avenue* and Beachwood Beach on 7/30/07. Morning samples collected between 6:31 and 6:59 am; Afternoon samples collected between 12:09 and 12:30 pm.
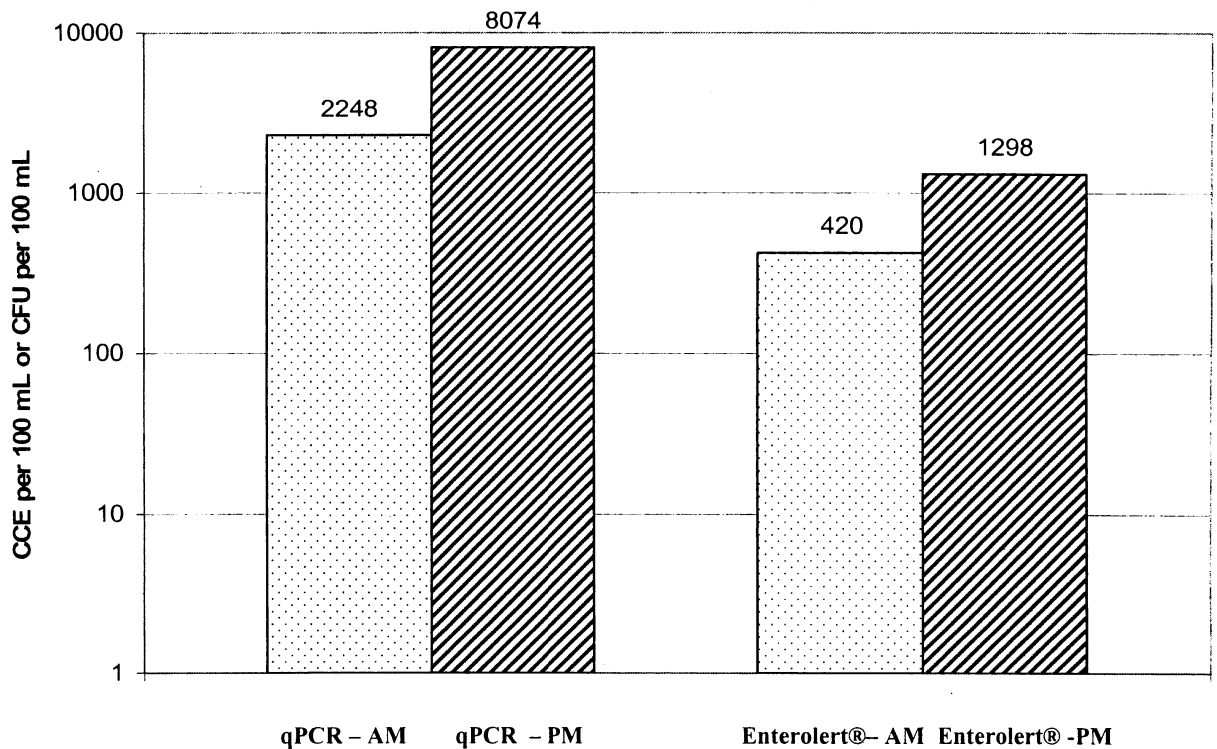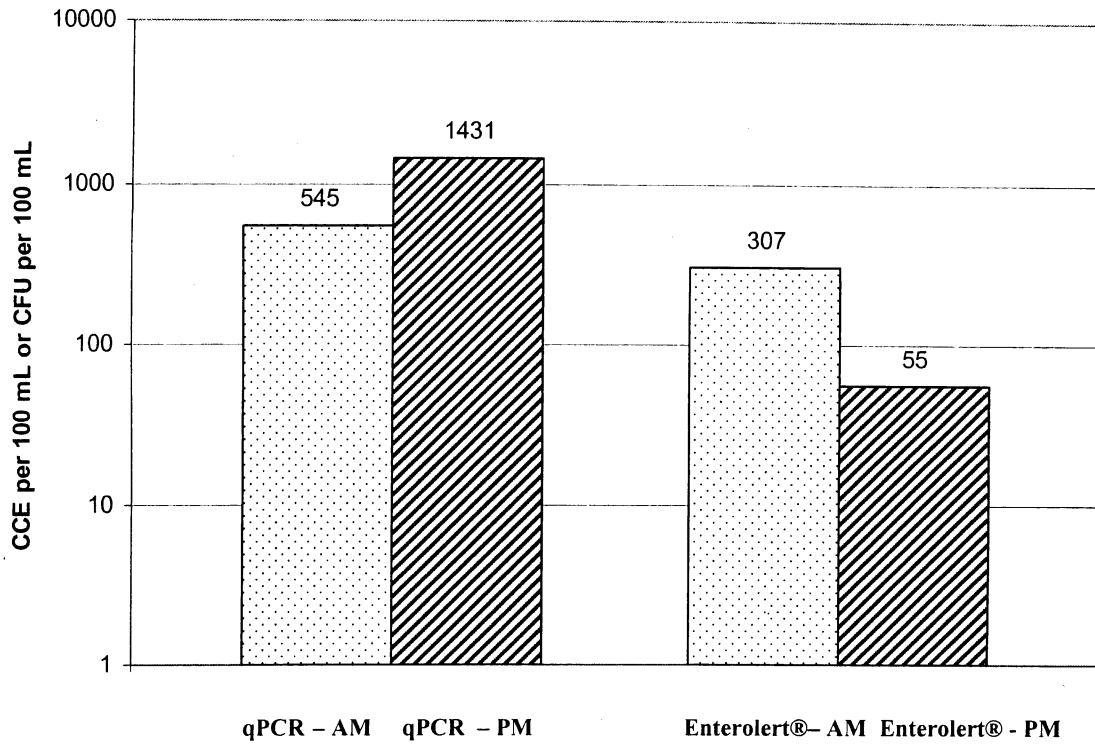
C) Windward Beach, Ocean County

Figure 5 c. Densities as geometric means (n=4) of *Enterococcus* using *qPCR Method 1606* (CCE) and Enterolert® (MPN/100 mL) on samples collected at Windward Beach on 7/30/07. Morning samples collected between 6:25 and 6:29 am; Afternoon samples collected at 1:00 pm.

Wade, et. al. (2006) found *Enterococcus* concentrations were higher in the afternoon in a recent study of bathing beach areas at the Great Lakes. There are many variables which could affect temporal bacterial densities including cloud cover, tidal stage (and water level as measured in feet above the mean low tide mark), wind direction and speed, water temperature, bather density, sampling location (bay versus ocean area) and sources of point and non-point source contributors of bacteria. Relative levels of bacteria in morning versus afternoon need to be evaluated on a site by site basis. Tidal stage, as measured by water level above mean low water mark, was seen as a significant determinant of *Enterococcus* density within the swimming areas (EPA 2005).

4.2 Spatial Factors Affecting *Enterococcus* Concentrations

Data from the EMPACT Study (EPA 2005) found that bacterial density was similar along a beach front transect as long as the distance from shore was similar. In this study, samples were collected at three stations along an approximate 80m transect in the morning and afternoon. While there were some differences in CCE and CFU/MPN values, the results were not significantly different along the transects (Figure 6a. and 6b.). There are many factors which may affect patterns of bacterial contamination along a transect. Tidal cycle, in-shore currents, point source discharges, and physical barriers in or near the bathing area (i.e., sandbar, pier, and reef) will be a factor at some beaches and not others. Determination of spatial variability was not a large part of the experimental design of this project. USEPA, state environmental agencies, and local Health Departments have guidelines established for determination of the most appropriate areas to sample to be representative and protective at a certain bathing beach. However, spatial variability is and important component to a beach monitoring program when evaluating a new monitoring technology or protocol.

4.3 Physio-Chemical Parameters Affecting *Enterococcus* Concentrations

Turbidity was low for all samples collected throughout the study (Tables 3 and 4). Only one station had a result which exceeded 10.1 NTUs (Recreation Center, Monmouth County, 30.2 NTUs) (Table 3a). This station also was characterized by the highest levels of *Enterococcus* by both qPCR and Enterolert ® (Table 3a).

Tables 3 and 4 summarize the physical/chemical data collected for this study. The data are arranged in a row based on the time sampled. Differences or trends of these measurements / observations can be evaluated versus changes in qPCR or MF/Enterolert® results over the course of the study.

There was no rainfall encountered during any of the sampling days of the 10 week study period, so differences in *Enterococcus* during wet weather conditions could not be evaluated. Salinity, water temperature, air temperature, cloud cover, wind direction and wind speed, and tidal cycle were recorded every time test samples were collected (data not presented). All of these parameters potentially can affect the concentrations of bacteria present. There was insufficient data or differences in results for these parameters to evaluate their effects on the *Enterococcus* measurements from this study.

4.4 qPCR Quality Control

There were no qPCR sample extracts which failed to meet the salmon DNA assay based acceptance criterion of 3 Ct units of the mean as outlined in the USEPA Draft 1606 qPCR methods.

There were 23 of 104 qPCR assays which exhibited a measurable amount of fluorescence during the qPCR analysis of the no template control (NTC) samples. The NTC contamination was not considered high enough to affect the quality of the associated test results. Two of the contaminated no template controls had C_T values of 35.59 and 35.64. The remaining had C_T values of 38.22 to 43.35. USEPA Draft Method 1606 suggests that any C_T value lower than 35 for an NTC should be repeated with a new master mix. However, all of the C_T values for the NTC in our study were greater than 35.

AE buffer was used as a filter blank and was processed with the test samples. One filtration blank was performed for every 6 test samples collected in this study. One of 44 filtration blanks collected for the Monmouth County samples and 3 of the 36 filtration blanks collected for the Ocean County samples exhibited a positive result for

6a. qPCR Transect Results
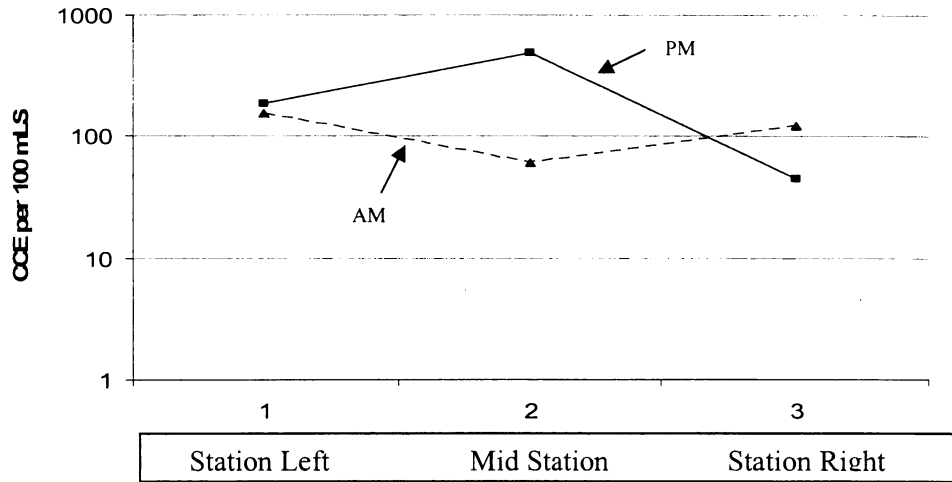
6b. Membrane Filtration Transect Results
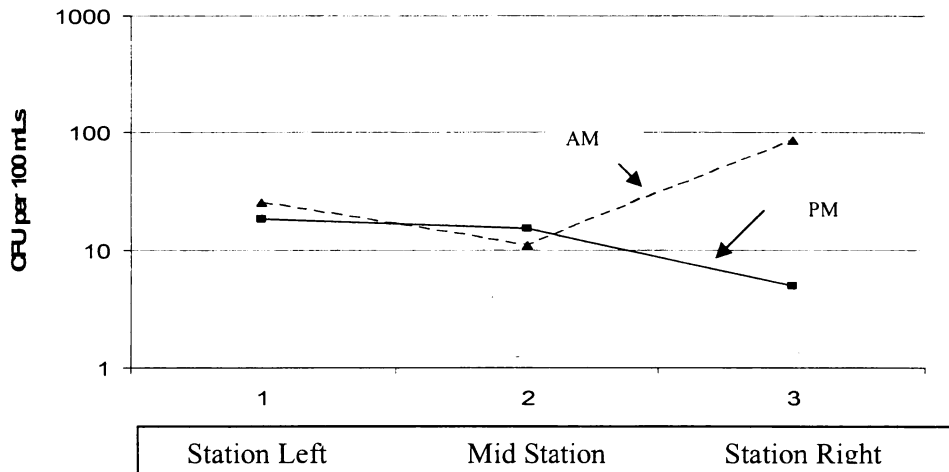
Figures 6a. and 6b. Comparison of qPCR results and MF results along a three station transect sampled at 8:00 am and 2:00 pm on August 6, 2007 at Myron/Wilson sampling area, Monmouth County.

Enterococcus during the qPCR analysis. The fluorescent signal was relatively low except one of the qPCR positive filtration blanks.

5.0 CONCLUSIONS

• qPCR was found to provide accurate and sensitive measurements of *Enterococcus* sp. concentrations and was performed in less than 4 hours per sample.

• Measurements of *Enterococcus* densities by the qPCR method and by the approved MF and Enterolert ® methods showed similar levels of between visit variability and within visit variability was generally higher for qPCR results from 20 bay and ocean recreational beaches and environmental sampling areas over a six week study period.

• A significant positive correlation was observed between the qPCR and both MF and Enterolert ® measurements of *Enterococcus* densities at all sampling areas, suggesting that the qPCR method has the potential to be used as a tool for beach management.

• The qPCR protocols are more complicated and the procedures are more sophisticated as compared to the traditional Membrane Filtration and defined substrate technology procedures (i.e., Enterolert ®). A higher level of expertise is needed to perform the analysis.

• There is a need is to collect epidemiological data in conjunction with qPCR data to help formulate appropriate risk values. Epidemiological studies are being performed by USEPA as part of the NEEAR program (National Epidemiological and Environmental Assessment of Recreation Water) study using qPCR data and the Method 1600 MF procedure. The objective of the NEEAR program is to evaluate the water quality at one or two beaches per year and ultimately obtain a new set of health and water quality data for the qPCR Enterococcus and *E. coli* protocols.

6.0 ACKNOWLEDGEMENTS

Table 3a. Summary of Chemical and Physical Parameters For Each Sampling Visit at Beaches and Bays in Monmouth County, New Jersey, June - August 2007.

Monmouth County

Sampling Week #	ARMY RECREATION BEACH					SURF BEACH					BROAD STREET					RECREATION CENTER				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
qPCR Geometric Mean	5	7	13	5	4	5	11	90	7	6	24	207	288	14	128	29	54	17394	1180	63
MF Geometric Mean	5	5	6	5	5	8	8	6	6	6	5	10	72	7	41	7	10	>600	242	33
Salinity	28.1	27.3	28.0	27.0	27.2	29.2	27.4	27.8	25.7	26.8	21.8	23.4	22.3	23.5	20.9	23.6	24.6	**24.5**	24	24.2
Turbidity	1.8	2.4	5.72	3.21	6.33	2	3.3	7.35	5.44	6.91	5.7	5.4	12	4.62	5.32	4.8	3.3	**30.2**	5.84	6.03
Air Temperature	75°	87°	63°	75°	65°	75°	87°	63°	75°	65°	73°	78°	64°	75°	65°	73°	78°	64°	75°	65°
Water Temperature	65°	76°	73°	75°	70°	65°	76°	73°	75°	70°	70°	75°	75°	75°	72°	70°	75°	75°	75°	72°
Precipitation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tidal Cycle	high	low	low	low	low	high	low	low	low	low	high	low	low	low	low	high	low	low	low	low
Wind Direction	SW	W	N	SW	E	SW	W	N	SW	E	NW	SSW	NNW	S	E	NW	SSW	NNW	S	E
Cloud Cover	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Time Collected	8:20	8:30	8:30	8:20	8:20	7:40	7:30	7:45	7:30	7:30	7:30	7:30	7:30	8:40	7:30	9:15	9:15	9:10	10:10	9:08

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs
ND = No Data
S = South, W= West, N= North, E= East
qPCR and MF/Enterolert were rounded to the nearest whole number
Time Collected = All AM
Need to Verify Rec. Center Salinity and Turbidity
Sampling Week 1 = 6/25/07; Week 2 = 7/9/07; Week 3=7/23/07; Week 4=8/6/07; Week 5=8/20/07

Table 3b. Summary of Chemical and Physical Parameters For Each Sampling Visit at Beaches and Bays in Monmouth County, New Jersey, June – August 2007.

Monmouth County

Sampling Week#	Cedar Ave					Village Beach Club					Myron/Wilson Bay					Newark Ave.				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
qPCR Geomean	17	26	197	6	314	76	186	101	10	51	43	389	955	59	331	4	14	34	6	91
MF Geometric Mean	6	14	59	5	38	5	6	29	6	20	29	97	307	10	11	5	7	7	7	10
Salinity	29.9	29.5	28.9	29.7	28.9	30	28.3	28.7	29.1	28.9	28.3	28.6	28.4	28	26.8	30.2	30	28.6	29.2	28.4
Turbidity	8.2	7.3	18	4.89	7.25	3.6	3.9	11.2	4.37	10.8	3.4	2.7	5.68	10.1	6.91	1.4	1.3	4.38	1.67	5.08
Air Temperature	70°	80°	64°	75°	62°	70°	80°	64°	75°	62°	70°	80°	60°	75°	65°	70°	80°	62°	72°	62°
Water Temperature	68°	68°	71°	75°	70°	68°	68°	71°	75°	70°	70°	70°	70°	75°	70°	71°	68°	68°	75°	70°
Precipitation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tidal Cycle	high	low	low	low	low	high	low	low	low	low	high	low	low	low	low	high	low	low	low	low
Wind Direction	SW	WSW	N	S	ENE	SW	WSW	N	S	ENE	NW	W	WNW	SW	NE	WSW	W	NNW	S	ENE
Cloud Cover	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Time Collected	8:55	8:45	8:55	9:00	8:45	8:40	9:00	8:40	8:45	9:00	7:37	7:42	7:37	7:38	7:37	9:05	7:20	9:20	9:05	7:26

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs
ND = No Data
S = South, W= West, N= North, E= East
qPCR and MF results were rounded to the nearest whole number
Time Collected = All AM
Need to Verify Rec. Center Salinity and Turbidity
Sampling Week 1 = 6/25/07; Week 2 = 7/9/07; Week 3=7/23/07; Week 4=8/6/07; Week 5=8/20/07

Table 3c. Summary of Chemical and Physical Parameters For Each Sampling Visit at Beaches and Bays in Monmouth County, New Jersey, June – August 2007.

Monmouth County

	Brown Ave					The Terrace				
Sampling Week #	1	2	3	4	5	1	2	3	4	5
qPCR Geomean	5	7	70	3	62	8	3	3	5	79
MF Geometric Mean	6	5	153	11	20	6	6	5	5	42
Salinity	30.2	28	27.7	29.4	29.4	30.1	29.6	29.3	29.8	28.8
Turbidity	1.1	1.5	7.12	1.4	6.55	1.1	1.1	3.95	2.04	4.98
Air Temperature	70°	80°	62°	72°	62°	70°	80°	62°	72°	62°
Water Temperature	71°	68°	68°	75°	70°	71°	68°	68°	75°	70°
Precipitation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tidal Cycle	high	low	low	low	low	high	low	low	low	low
Wind Direction	WSW	W	NNW	S	ENE	WSW	W	NNW	S	ENE
Cloud Cover	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Time Collected	8:35	7:55	8:40	8:30	8:05	8:00	8:15	8:30	8:15	8:30

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs
ND = No Data
S = South, W= West, N= North, E= East
qPCR and MF results were rounded to the nearest whole number
Time Collected = All AM
Need to Verify Rec. Center Salinity and Turbidity
Sampling Week 1 = 6/25/07; Week 2 = 7/9/07; Week 3=7/23/07; Week 4=8/6/07; Week 5=8/20/07

Table 4a. Summary of Chemical and Physical Parameters For Each Sampling Visit at Beaches and Bays in Ocean County, New Jersey, June – August 2007.

Ocean County

Sampling Week #	Broadway					Windward Beach					Sheridan Avenue					Central Avenue				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
qPCR Geometric Mean	3	6	10	5	133	17	154	152	527	243	18	4	90	-2	7	289	1286	2526	3408	5680
Enterolert Geometric Mean	14	7	9	9	14	12	12	7	300	79	12	7	6	5	10	73	51	527	521	75
Salinity	ND	34	34	34	32	ND	22	25	17	23	ND	35	35	34	32	ND	16	18	12	15
Turbidity	4.2	2.4	10	3.5	1.1	3.3	5.3	4.9	1.1	5.8	3.4	4.7	5	7.2	2.4	3.4	6.7	5.7	5.4	4.5
Air Temperature	73°	62°	78°	73°	70°	71°	60°	76°	71°	70°	73°	56°	72°	70°	75°	70°	59°	75°	71°	74°
Water Temperature	68°	65°	65°	65°	70°	74°	67°	76°	75°	75°	70°	62°	70°	70°	70°	68°	70°	74°	73°	73°
Precipitation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Tidal Cycle	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wind Direction	W	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm	S	N	Calm	Calm
Cloud Cover	PC	S	C	C	Cl	PC	S	C	C	C	PC	S	PC	C	C	S	S	S	C	C
Time Collected	7:01	7:02	6:58	6:57	6:42	6:19	6:27	6:22	6:28	6:20	6:28	6:30	6:25	6:35	6:31	6:28	6:35	6:31	6:33	6:35

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; Salinity=ppth
ND = No Data
S = South, W= West, N= North, E= East, S=Sunny, C=Cloudy, PC= Partly Cloudy
qPCR and Enterolert® were rounded to the nearest whole number
Time Collected = All AM
Need to Verify Rec. Center Salinity and Turbidity
Sampling Week 1 = 6/18/07; Week 2 = 7/2/07; Week 3=7/16/07; Week 4=7/30/07; Week 5=8/13/07

Table 4b. Summary of Chemical and Physical Parameters For Each Sampling Visit at Beaches and Bays in Ocean County, New Jersey, June - August 2007.

Ocean County

Sampling Week #	Anglesea Avenue					Avon Road West					Money Island					Beachwood Beach				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
qPCR Geomean	13	70	333	2581	58	92	250	287	2560	354	34	172	237	1256	2076	495	494	706	2069	360
Enterolert Geomean	7	12	19	385	48	13	37	69	462	14	8	12	76	404	164	75	68	117	405	32
Salinity	ND	18	20	10	18	ND	13	14	5	13	ND	16	17	8	15	ND	12	12	5	10
Turbidity	4	4.8	6.6	4.2	3.5	4.5	4.9	5	1.4	4.7	3.6	5.3	6	4.4	6	5.2	6.5	5.1	4	3.8
Air Temperature	80°	59°	75°	71°	74°	80°	59°	75°	71°	74°	70°	59°	75°	70°	74°	80°	59°	75°	71°	74°
Water Temperature	77°	70°	74°	70°	73°	76°	70°	74°	68°	73°	68°	70°	74°	73°	73°	73°	70°	74°	68°	73°
Precipitation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Tidal Cycle	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wind Direction	calm	N	N	calm	calm	calm	N	N	calm	calm	calm	NE	N	calm	calm	calm	N	N	calm	calm
Cloud Cover	Clear	S	S	C	C	Clear	S	S	C	C	S	S	S	C	C	Clear	S	S	C	C
Time Collected	noon	7:25	7:27	7:26	7:24	11:45	7:08	7:07	7:08	7:07	6:15	6:09	6:27	6:25	6:26	11:40	7:03	7:01	6:58	6:54

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; Salinity=ppth
ND = No Data
S = South, W= West, N= North, E= East, S=Sunny, C=Cloudy, PC= Partly Cloudy
qPCR and Enterolert® were rounded to the nearest whole number
Time Collected = All AM
Need to Verify Rec. Center Salinity and Turbidity
Sampling Week 1 = 6/18/07; Week 2 = 7/2/07; Week 3=7/16/07; Week 4=7/30/07; Week 5=8/13/07

Table 4c. Summary of Chemical and Physical Parameters For Each Sampling Visit at Beaches and Bays in Ocean County, New Jersey, June – August 2007.

Ocean County

Sampling Week #	Ocean Area 1					Bay Beach				
	1	2	3	4	5	1	2	3	4	5
qPCR Geomean	3	5	16	10	4	3	5	40	21	48
Enterolert Geomean	5	6	6	5	10	5	8	7	25	12
Salinity	ND	34	34	34	32	ND	32	33	31	31
Turbidity	4.4	1.4	4.6	1.1	1.8	3	6	5.7	7.1	6
Air Temperature	77°	75°	74°	70°	75°	70°	77°	80°	77°	82°
Water Temperature	70°	70°	70°	70°	70°	72°	71°	70°	70°	71°
Precipitation	No	No	No	No	No	No	No	No	No	No
Tidal Cycle	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wind Direction	calm	calm	calm	calm	calm	calm	calm	calm	calm	calm
Cloud Cover	C	Clear	C	C	C	clear	clear	clear	clear	C
Time Collected	8:01	8:21	8:30	7:57	9:03	7:42	7:28	8:15	7:37	730

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; Salinity=ppth
ND = No Data
S = South, W= West, N= North, E= East, S=Sunny, C=Cloudy, PC= Partly Cloudy
qPCR and Enterolert® were rounded to the nearest whole number
Time Collected = All AM
Need to Verify Rec. Center Salinity and Turbidity
Sampling Week 1 = 6/18/07; Week 2 = 7/2/07; Week 3=7/16/07; Week 4=7/30/07; Week 5=8/13/07

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