COMPARISON OF *ENTEROCOCCUS* MEASUREMENTS IN MARINE BEACH AND BAY SAMPLES BY QUANTITATIVE (REAL-TIME) POLYMERASE CHAIN REACTION, MEMBRANE FILTRATION AND ENTEROLERT

REPORT EXECUTIVE SUMMARY

1. INTRODUCTION

►Real Time or quantitative Polymerase Chain Reaction (qPCR) is a technique used to amplify or copy a specific region of DNA. PCR's power comes from the fact that every animal, bacteria or virus possesses sequences (or portions of their bodies' building blocks called DNA) that are unique and present only in its own species. The development of this Nobel Prize winning technique by Kary Mullis in the 1980's resulted in the application of many new techniques in molecular biology. Automation of PCR through the use of Thermal Cyclers has been the key to the rapid use and multiple applications of this technology throughout the science community.

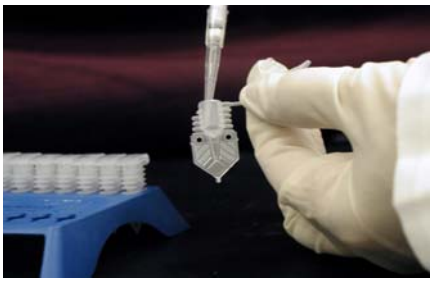
2. BACKGROUND

►Previous health and epidemiological studies by US Environmental Protection Agency (USEPA) have demonstrated that the amount of the bacterial genus *Enterococcus* in both marine and freshwater samples is directly correlated with gastroenteritis illness rates in swimmers exposed to these waters.

►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 indicator organisms for the presence of the bacterial and viral pathogens found in fecal material and/or the intestines of warm blooded animals.

►Currently, approved methods for measuring concentrations of *Enterococcus* and *E. coli* in recreational waters include membrane filtration and most probable number tests. Although many of these methods have been used for decades, results are typically not available for 24 hours.

►At best, a decision regarding safe beach usage is made using one day old information or is not made until after a confirmation test which can take up to 72 hours after the initial sample is collected. Most qPCR tests can provide results in as little as 4 hours. Because microbial water quality can change rapidly, testing based on indicator organisms (that requires 24 hours to obtain results), may result in unnecessary beach closings or exposure of swimmers to water of poor microbial quality.



►Congress passed an amendment to the Clean Water Act called the Beaches Environmental Assessment and Coastal Health (BEACH) Act. The BEACH Act requires the USEPA to conduct research in support of new recreational water criteria. Because qPCR methods provide a faster assessment of water quality, they have the potential to improve decision making for county and state personnel involved in beach management decisions.

3. qPCR COMPARSION STUDY IN MARINE BEACHES AND BAYS, 2007

►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.

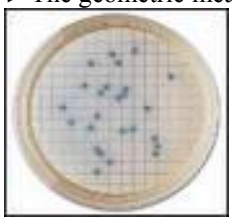
►Ocean and bay samples with varying levels (based on historical data) of expected microbial densities were sampled 10 times between June 18 and August 20, 2007.

►Samples were collected in thigh deep water approximately 0.3 meters from the surface.

►Cell densities of the fecal pollution indicator genus, *Enterococcus*, were determined by qPCR, as well as two conventional 24 hour test methods (membrane filtration [MF] and Enterolert). Over 1000 samples were analyzed.

4. RESULTS

►The geometric means ranged from 6.8 to 188 calibrator cell equivalents (CCE) by qPCR analysis compared to 5.2 to 64.9 colony forming units (CFU) by MF analysis in Monmouth County Beach/Bay Samples (100 mL samples).



►The geometric means from the samples collected in Ocean County were 6.6 to 1785 CCE by qPCR compared to 6.2 to 150 CFU by Enterolert (N=200).

► 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.

►Up to a 12 fold higher amount of enterococci was detected by qPCR. However, the 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 0.79.

5. CONCLUSIONS

►The results of the study indicate that qPCR shows promise as a beach monitoring tool which can provide results in as little as 4 hours.

►While qPCR generally provided higher results, there was a strong correlation between qPCR results and conventional enterococci methods.

►Additional data are needed to further refine qPCR technology for routine use at marine bathing beaches, including an interlaboratory method validation study and an epidemiological study using qPCR water quality data. USEPA Region 2, NJDEP, and the Ocean and Monmouth County Health Departments are planning a follow up study in 2008 to further delineate spatial and temporal trends of enterococci measurements using qPCR in Ocean and Monmouth County beaches.