Fact Sheet

Recreational Bathing Beach Demonstration Project Using qPCR

Ocean County, New Jersey

What is real time Polymerase Chain Reaction or qPCR?

Real time Polymerase Chain Reaction (qPCR) is a technique used to amplify the number of copies of a specific region of DNA. DNA stands for Deoxyribonucleic acid. DNA is a nucleic acid present in the cells of all living organisms. It is often referred to as the "building blocks of life" since DNA encodes the genetic material which determines what an organism will develop into. Most living things use a chemical called polymerase to repair or build new copies of genetic code. qPCR is sometimes called "molecular photocopying" because the polymerase chain reaction is a fast and inexpensive technique used to amplify or copy a small segment of DNA from a target organism. PCR's power comes from the fact that every animal, bacteria or virus possesses DNA that is unique and present only in its own species. Automation of PCR through the use of Thermal Cyclers has been the key to the rapid use and multiple applications of qPCR.

How is bathing beach water quality measured and what are the limitations?

EPA requires that marine recreational waters across the United States be monitored routinely for *Enterococcus* bacteria. While enteroccos bacteria is not pathogenic, it is considered to be an indicator of other organisms that may cause illnesses. The current approved laboratory method for measuring concentrations of Enterococci bacteria in recreational waters uses the membrane filter technique and take 24 hours to produce results. 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 poor microbial water quality. Basically you are making decisions on water quality based on conditions that occurred 24 hours ago.

How did we get to this point and how does qPCR fit in?

Congress passed an amendment to the Clean Water Act, the Beaches Environmental Assessment and Coastal Heath (BEACH) Act. One main objective of the Beach Act required EPA to conduct research to provide the support of new criteria for recreational water. EPA expects to publish new criteria in the Federal Register by October 2012 (public comments by February 2012). Epidemiological studies have been conducted using membrane filtration and qPCR data to begin formulation of new water quality criteria for pathogens. Although some technical issues continue to be evaluated, the use of qPCR assays have shown promise as an alternative technology to monitor microbial water quality at recreational beaches.

How does qPCR Work?

DNA replicates itself as part of many normal biological processes and qPCR imitates and speeds up this process in the laboratory. A thermal cycler is used to rapidly heat and cool samples to facilitate this process and uses spectrophotometry to produce results. Listed below is a brief and simplified overview of the process:

- Bacteria in the water sample are captured on a filter and mixed with reagents that are conducive to DNA replication.
- Fluorescent probes that adhere to the new DNA copies are added, and the sample is subjected to repeated heating and cooling cycles to double the amount of target DNA every 2-3 minutes.
- A computer records the amount of fluorescence in real time to estimate how much DNA, and how many cells were present in the original sample.

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What is the Endpoint Measured Using qPCR?

- Conventional Membrane Filtration (MF) techniques reports results as Colony Forming Units (CFU). CFU's are masses of one culturable target bacteria cell that can be observed after a set amount of culturing time (usually 18-24 hours). qPCR detects fundamentally different targets (DNA sequences) than culture based methods. Calibrator Cell Equivalents (CCE) are used as the endpoint for qPCR. CCE involves an arithmetic formula to determine target sequence quantities in DNA extracts from test samples relative to those in similarly prepared DNA extracts from calibrator samples containing a known quantity of target organism cells.

- In 2007, a comparison study of 20 beaches, embayments, and environmental sampling areas was conducted in Ocean and Monmouth Counties in NJ. Overall, there was good agreement between qPCR and the conventional membrane filtration and Enterolert Methods with an overall correlation coefficient (r) of 0.71. Based on previous comparison studies, the primary contact standard for qPCR for this project will be 120 CCE./100 mL.

Has any data been collected using qPCR at NJ Beaches to this point?

- Yes, a collaboration of USEPA Region 2, NJDEP, and Ocean and Monmouth County Health Departments have been collecting qPCR data since 2007 at various marine beaches, embayments and environmental sampling areas.

- Results have indicated that qPCR can be a fast and reliable tool to monitor water quality at New Jersey marine beaches.

- Studies were performed to evaluate precision and correlation between qPCR and membrane filtration technologies. Evaluation of spatial and temporal data, interlaboratory variability, and different qPCR thermal cyclers were included in the experimental designs.

- Additional Information on the 2008 and 2007 studies can be found at the following websites:

  http://www.epa.gov/region02/water/oceans/2008Report_QPCR_NJ_Final_Jan2010.pdf

  http://www.epa.gov/region2/water/oceans/june2008resultsfactsheet.pdf

What is planned for the 2011 Demonstration Project?

- Four marine bathing beach stations will be sampled in Ocean County weekly beginning in July, 2011.

- Split samples will be collected for both qPCR and membrane filtration analysis.

- qPCR results will be available and posted on the same day that samples are collected. A swimming advisory will be issued when beaches exceed the qPCR standard on the first sample. Check www.njbeaches.org. Results will be posted by 2:00 p.m.

- qPCR samples will be analyzed in duplicate and both results must average greater than 120 CCE per 100 mL to trigger a resampling event in the afternoon. Results of any resampling events will be posted on www.njbeaches.org by noon the following day following the original sampling event.

- Comparison of MF and qPCR results will be evaluated throughout the study as well as other qPCR performance statistics such as number of false positives, false negatives, amplification efficiency, and inhibition of PCR amplification.

For more information please contact Jim Ferretti, USEPA Region 2 (ferretti.jim@epa.gov) or Virginia Loftin, NJDEP (virginia.loftin@dep.state.nj.us) or visit www. njbeaches.org