EXECUTIVE SUMMARY

Public health officials routinely monitor fecal indicator bacteria levels to assess beach water quality. However, present laboratory processing methods require approximately 24 hours for completion and swimmers may be exposed to poor water quality during this time. New, more rapid assessment methods that would allow for same-day water quality warnings are under development, but require rigorous independent testing. This study evaluates new methods being developed by six research groups.

Three research groups utilized Quantitative Polymerase Chain Reaction (QPCR), a genetic method that quantifies a DNA target via a fluorescently tagged probe. A fourth research group used Transcription-mediated Amplification (TMA), another genetic method that relies on a fluorgenic probe for quantification, but targets RNA rather than DNA. The fifth group used Dual-Wavelength Fluorimetry (DWF), a defined substrate (DS) method that utilizes the same fluorogenic substrates as traditional IDEXX methods, but coupled with an advanced optical detection system to produce results after an incubation period of only a few hours. The sixth group used an immunological dipstick method that is designed to provide a binary answer about whether bacterial concentrations in a sample exceed California's single-sample standard. Although the study focused on methods for enumerating enterococci, two of the groups using a QPCR method and the group using DWF also measured *E. coli* in their test samples. The immunological dipstick method targeted only *E. coli*.

Testing involved processing 18 blind samples in triplicate and comparing these results to those obtained by 5 local laboratories that processed the same samples using traditional methods. Test samples included both natural and laboratory-created samples, ensuring method evaluation over a range of concentrations, matrices, and interferences. Each research group processed nine samples consisting of clean seawater inoculated with three different concentrations of laboratory culture, sewage, or urban runoff. Six were ambient water samples from locations throughout southern California and three were blanks. Each method's performance was evaluated with respect to individual sample and average concentration across replicates, the State's water quality standard, and variability among replicates by comparing new method results to results obtained through traditional methods. These evaluations were integrated into an overall assessment to determine if management decisions based on new method results would have been the same as decisions based on traditional methods.

Results from two of the QPCR methods and the TMA method were more than 80% accurate with respect to the State standard for enterococci. These methods also proved consistent in terms of effect on beach management decisions, concurring with decisions based on EPA approved methods results for more than 75% of the samples. Results for one of the *E. coli* QPCR methods were even more promising, with 90% agreement about beach management decisions. This is comparable to the rate of agreement between the two traditional methods used in this study.

The new methods measure different bacterial properties than EPA approved methods and are not expected to produce completely equivalent results. Readiness of the new methods for routine use is a subjective determination that should involve balancing equivalency between new and traditional water quality monitoring methods, with the desire to incorporate new rapid methods into a beach water quality warning system that is presently handicapped by extended sample

processing time. California's Beach Water Quality Workgroup is a collection of water quality specialists that was asked to define the necessary level of equivalency before recommending State certification of these methods. The workgroup identified six applications for rapid indicators, established desired levels of equivalency for each application, and determined that the QPCR and TMA methods appear ready for use in several, but not all, of the applications. However, the workgroup also identified the desirability for additional testing focused on ambient samples and conducted by personnel from local laboratories, rather than the method developers. This additional testing is now ongoing.