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EXECUTIVE SUMMARY

Quantitative polymerase chain reaction (qPCR) methods have demonstrated great potential for changing how recreational water quality is monitored, enhancing the speed to result, specificity, and sensitivity compared to traditional culture-based methods. There are two main applications for qPCR in recreational water quality monitoring. The first is a more rapid measurement of fecal indicator bacteria (FIB) to facilitate timely public notification when beach water is contaminated or a contamination event has passed. For example, U.S. EPA's 2012 revised recreational water quality criteria allows routine use of qPCR to measure Enterococcus spp. for rapid beach monitoring. The second application is to identify sources of fecal pollution using host-associated source markers. In this case, the California Microbial Source Identification Manual recommends a suite of validated qPCR-based methods to measure fecal source identification markers for identifying sources of fecal pollution in ambient waters.

The ability to transition these applications to practical management use relies on three aspects. The first is feasibility of technology transfer. Despite continued advancements in the development of qPCR-based methodologies, these tools are still primarily employed by expert-users in research labs. Furthermore, evaluative studies of the efficacy of transferring qPCR technology to public agencies responsible for routine monitoring have been limited to relatively small demonstration projects of short duration. Regional scale assessment of these methods across a variety of laboratories with varying levels of familiarity with qPCR is necessary to assess whether this technology may be realistically transitioned to end-users. Regulatory issues are the second important aspect affecting transition success. Before granting

approval of qPCR quantification of enterococci as a replacement for culture-based monitoring of FIB, the U.S. EPA encourages a site-specific analysis of qPCR's performance. An implementation threshold (e.g. for beach posting) also needs to be established based on demonstrated consistent and predictable relationship with an EPA-approved benchmark method such as culture-based method. The third aspect involved in transition success is technical. While the qPCR-measured host-associated markers have been used extensively as a diagnostic tool to determine sources of fecal contamination within watersheds, there is no framework for interpreting marker results from routine monitoring to prioritize remediation actions amongst multiple watersheds.
The Microbiology Committee of the Southern California Bight '13 Regional Monitoring Program focused its efforts on addressing questions surrounding the efficacy of employing qPCR methods in southern California with three study goals:

1. Assess the ability of laboratories conducting routine water quality monitoring in the southern California region to employ qPCR methods.
2. Assess comparability of results between qPCR and traditional culture for measuring Enterococcus spp. at southern California beaches.
3. Determine the management value of regional monitoring for a human fecal source marker HF183 to prioritize sites for remediation.

To address the first goal, we evaluated the ability of eight water quality monitoring labs to produce comparable results using qPCR. The evaluation consisted of three phases. The first was a training workshop in which local agencies learned how to perform the qPCR methods. The second was an intercalibration study conducted two months post-training designed to assess whether the proficiency demonstrated by participants at the completion of training persisted after they returned to their home laboratories. The third phase was a long-term assessment of laboratory performance over three subsequent years. Technology transfer of qPCR to local monitoring laboratories was found to be feasible for both the enterococci and the HF183 qPCR methods, with all participating labs demonstrating proficiency in performing the methods during the intercalibration phase. However, some laboratories did not sustain their proficiency over the 3-year implementation, as indicated by a lack of repeatability and inability to consistently produce acceptable standard curves. The higher failure rate in the long-term evaluation was laboratory specific and appeared attributable to turnover of laboratory personnel. Our results illustrate the need for formal lab training and accreditation programs for qPCR methods that includes standardized training, evaluation, and certification of laboratories performing qPCR methods for water quality monitoring.

To address the second goal, we evaluated the level of agreement between enterococci measured in parallel by both culture and qPCR methods at 36 southern California beaches following a formal procedure outlined in an EPA technical supporting material (TSM) document. Approximately 50 samples were collected at each beach in dry weather during both summer and winter seasons. Only one site in summer and three sites in winter achieved the level of agreement required by EPA to allow replacement of the culture-based method with qPCR for posting the beach. Matrix interference, in which constituents in environmental waters interfere with the qPCR chemistry leading to underestimation, was not an obstacle for qPCR equivalency, nor was laboratory performance an impediment. The lack of demonstrable agreement was largely due to enterococci concentrations at most sites being low; even with two years of weekly sample collection, we were not able to achieve the requisite number of samples in the quantifiable range for most beaches. In addition, the useable measurements were mostly at the low end of the quantification range for both methods. This produced a limited range of values, contributing to high method variability and thus, low correlation. The few sites that did meet the EPA requirements were ones that had concentrations that were frequently above the beach warning thresholds. This suggests that some modification of the EPA protocols is necessary if qPCR is to be adopted at sites that are typically clean

and subject only to transient pollution events, which are the sites where the speed advantage of qPCR is most valuable for improved public health protection.
To address the third goal, we conducted a regional assessment of human marker prevalence among drainages that discharge to the southern California ocean. Approximately 50 samples from each of 22 southern California coastal drainages were collected under summer dry weather conditions. An additional 50 samples were targeted from each of 23 drainages during wet weather, although a drought during this period prevented us from achieving the targeted number of samples at all wet weather sites. Samples were analyzed for the HF183 human fecal marker, which was found to be ubiquitous across the region; it was present at all but two sites in dry weather and at all sites during wet weather. There was considerable difference in the extent of human fecal contamination among sites. While site rankings remained consistent regardless of whether ranking was based on frequency of HF183 detection or on average concentration of HF183, site ranking differed greatly between dry and wet weather. Site ranking also differed greatly between if based on HF183 and if based on enterococci, which, unlike HF183, does not distinguish between human and non-human fecal contamination. Although, additional work is needed to interpret results regarding human health impacts, these results illustrate the management value of HF183 as a monitoring tool to enable more effective prioritization of sites for remediation.

Full Text

http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/1005_B13_BightShorelineMicrobiology.pdf