Comparison of rapid QPCR-based and culture-based methods for enumeration of *Enterococcus* sp. and *Escherichia coli* in recreational waters

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Abstract

Recreational water quality is currently assessed by measuring bacterial indicators using United States Environmental Protection Agency (EPA) approved culture-based methods that require 18 to 96 hours for results, limiting the immediacy of public health warning systems. Quantitative polymerase chain reaction (QPCR) methods that can be completed in less than two hours have been developed, but measure a different endpoint that could yield different water quality conclusions than the existing EPA approved methods they are intended to replace. Here we present two studies in which samples were processed simultaneously using QPCR- and culture-based methods of enumeration for *Enterococcus* sp. and *Escherichia coli* to assess how frequently disparities occur between these classes of methods. The first study involved processing 54 blind samples, in which QPCR analysis was conducted by developers of the assays. The second study involved 163 samples processed by personnel from a State certified microbiology laboratory with little previous experience with QPCR. The correlation between QPCR- and culture-based methods was 0.80 for *Enterococcus* and 0.84 for *E. coli*, which is only slightly less than the 0.92 correlation observed between the two culture-based methods. There were no false positives on blank samples and repeatability between replicates was as high for QPCR as it was for the culture-based methods, even when performed by the laboratory without previous QPCR experience. The QPCR results would have led to the same beach management decision as the culture-based methods for 88% of the samples: only slightly less than the 94% agreement in beach management decisions based on the two culture-based methods. The samples for which there was disagreement suggest a slight bias toward underestimation for the QPCR-based method. This underestimation might have been due to amplification inhibition or greater target specificity in the QPCR assays. While there is still a need to understand these minor differences, the high level of agreement should facilitate transition from culture-based to rapid QPCR-based methods.

Introduction

Presently, fecal indicator bacteria are measured to assess recreational water quality using one of three EPA approved methods: membrane filtration, multiple tube fermentation, or defined substrate technologies (DST). These methods are widely accepted because of their relative ease of use, low cost, and demonstrated relationship to health risk. However, the time required for sample processing ranges from 18 to 96 hours, with confirmation and verification steps taking even longer. Beach bacterial indicator concentrations have been shown to change substantially on time scales of less than a day (Boehm *et al*. 2002). Thus, contaminated beaches remain open during the laboratory processing period, and often the contamination event has passed by the time warnings are posted (Leecaster and Weisberg 2001).

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Advances in molecular techniques provide new opportunities to measure bacteria more rapidly (Haugland *et al*. 2005, Noble and Weisberg 2005, Layton *et al*. 2006, Bushon *et al*. 2009). While currently used methods rely on bacterial growth and metabolic activity, these new methods directly measure cellular attributes, such as genetic material or surface immunological properties. By eliminating the need for a lengthy incubation step, results are available in several hours, enabling managers to take action to protect public health (i.e., post warnings or close beaches) on the same day that water samples are collected. The most advanced of these new methods is QPCR, which has been found to perform well in epidemiological studies as a predictor of gastrointestinal illness risk in beachgoers (Wade *et al*. 2006, 2008).

While QPCR-based methods are promising, results may differ from those of the culture-based methods that they are intended to replace. Some differences may be related to quantificaction of a nucleic acid endpoints, which could potentially result in detection of bacterial fragments that would not have been measured by culture-based methods. Differences may also be related to chemical inhibition of genetic amplification or challenges in training personnel without previous experience using molecular methods. Acceptance of new methods by water quality professionals with a long history of using culture-based methods will depend on understanding the frequency and the underlying causes of these differences.

Whereas a number of studies have assessed the relative performance of the three most commonly used culture-based methods (Noble *et al*. 2003, Griffith *et al*. 2006, United States Federal Register 2006), there have been few comparisons between QPCR- and culture-based method performance. Here we compare performance of a QPCR-based method with two culture-based methods for *Enterococcus* sp. and one culture-based method for *E. coli*. We also quantify the effect of two different QPCR sample processing approaches and assess the performance of each when implemented by personnel from a State certified water quality laboratory that would be expected to employ these approaches, if adopted.

Methods

The study involved two types of testing in which water samples were simultaneously processed using QPCR-based and EPA approved culture-based methods. In the first test, the QPCR assays were conducted by the scientist who developed the method. In the second test, the sample processing and the QPCR analyses were conducted by personnel from a State certified water quality microbiology laboratory without previous QPCR experience to assess whether a more typical user could produce comparable results.

Study Design

The first study involved 54 blind samples consisting of triplicates of each of 18 different test samples. Nine of the eighteen samples were created by inoculating differing levels of fecal contamination into a seawater matrix. Six samples were natural ambient samples collected at shoreline locations with historically high concentrations of fecal indicator bacteria, including: Imperial Beach, San Diego, CA; Doheny State Beach, Dana Point, CA; Cabrillo Beach, Los Angeles, CA; Surfrider State Beach, Malibu, CA; Paradise Cove, Malibu, CA; and a freshwater sample from the Tijuana River, San Diego, CA. The last three samples were various types of blanks, consisting of sterile phosphate-buffered saline (PBS) pH 7.2, uninoculated offshore seawater, and 0.2 µm filtered offshore seawater.

The laboratory created samples were prepared using seawater collected from 18 km offshore of Newport Beach, CA, at a depth of 10 m, in an area known to be free from allochthonous fecal contamination. Three of these samples were inoculated with differing concentrations of laboratory cultures (*Enterococcus faecium*, *Enterococcus faecalis* and *E. coli*). Another three samples were inoculated with differing concentrations of primary wastewater influent from Orange County Sanitation District Plant #1 (OCSD; Fountain Valley, CA), and three were inoculated with differing concentrations of urban runoff collected from a Dominguez Channel storm sewer in Torrance, CA.

Sample processing for the culture-based methods was conducted by five local laboratories: OCSD, Orange County Public Health Laboratory, City of Los Angeles, Los Angeles County Sanitation District, and the City of San Diego, using methods employed in their routine water quality monitoring programs. For *Enterococcus* sp., sample processing included the Enterolert™ (IDEXX Laboratories, Inc., Westbrook, ME) DST and the EPA Method 1600 membrane filtration (Frahm and Obst 2003, Messer and Dufour 1998). For *E. coli*, only Colilert-18® (IDEXX) DST was used.

Testing took place June 21-23, 2005. Samples were created or collected between 6 and 9 a.m. each day and distributed to all laboratories no later than 11 a.m. Samples were all processed starting at the same time in all laboratories and in numbered order to minimize any concentration differences that might have developed from degradation during sample transport or laboratory holding. Further details are available as to other results from this study in Griffith *et al*. 2007.

The second study was conducted from February through July 2006 and involved OCSD microbiologists processing 163 samples using both culture-based and QPCR methods. Of these samples, 137 were ambient samples collected from 41 locations that are part of the microbiologists' typical weekly monitoring efforts. The remaining 26 samples were seawater spiked with primary sewage influent (19 samples), or secondary effluent (6 samples; Table 1). All samples were processed in duplicate using QPCR, EPA Method 1600, Enterolert, and Colilert-18. Forty-four samples were processed using bead-beating followed by a commercial DNA extraction kit; these samples were not included in the final data analysis. Therefore, the comparison of the QPCR data was only with samples that were processed using bead beating.

Ambient water samples were collected from five location types: open ocean beaches distant from creeks that drain land-based runoff (Open Ocean Beach); open ocean beaches near storm drains (Open Beach Near Drain); enclosed embayment beaches (Enclosed Beaches); locations within storm drains; and wet weather samples from open ocean beaches (Wet Weather; Table 1). Sewage spiked samples were created by inoculating clean ocean water with varying concentrations of either primary sewage influent or secondary sewage effluent (Table 1). Clean ocean water was collected at a location 11 km offshore of Newport Beach, CA. Sewage was obtained from OCSD wastewater stream. Following inoculation, sewage spiked samples were stirred for a minimum of 15 minutes using a magnetic stirring plate.

Sample Processing for QPCR

The QPCR assays were a proprietary method developed for *Enterococcus* sp. targeting the multiple copy 23S rRNA gene in an approach similar to that outlined by Ludwig and Schleifer (2000). The *E. coli* assay targets the single copy *uidA* gene as discussed in Frahm and Obst (2003). Scorpion™ primer/probe technology (DxS, Ltd., Manchester, UK) was utilized for assay development. Primer and probe sequences for *Enterococcus* sp. and *E. coli* QPCR assays are licensed and sold by Cepheid as proprietary Total *Enterococcus* and *E. coli* SmartBeads (Cepheid, Inc., Sunnyvale, CA). A *Lactococcus* bead containing 100,000 cells, also marketed by Cepheid, was used as a specimen processing control (SPC) to assess inhibition for each analysis.

Samples were processed on a six-place filtration manifold and vacuum pump assembly with Pall disposable filter funnels (Pall Corp., East Hills, NY). The mixed-ester cellulose filters that had been provided with the funnels from the manufacturer were

Table 1. Type and number of samples analyzed for *Enterococcus* sp. and *E. coli* analyses during the second study.

Sample Type	Number of Samples Collected	Samples Included in Data Analysis for QPCR	Samples Analyzed using Culture-Based Methods
Open Ocean Beach	44	33	36
Ocean Beach Near Drain	18	18	18
Enclosed Beach	22	22	22
Storm Drain	29	29	29
Wet Weather	25	25	25
Open Beach Spiked with Primary Sewage Influent	19	19	19
Open Beach Spiked with Secondary Sewage Effluent	6	6	6
Total	163	152	155

replaced with 47-mm diameter, 0.45-µm pore size polycarbonate filters (HTTP; Millipore Corp., Bedford, MA). Each 100-ml sample (measured using a sterile 50-ml conical tube) was filtered within 30 minutes of receipt. Sample filtration was conducted until no further moisture appeared on the filter. Each filter was subsequently rinsed with a small volume (~20 ml) of PBS, which was also filtered to visible dryness.

For the first study, replicate filters were processed twice, using either bead beating only or bead beating followed by a full DNA extraction. Filters were immediately removed from the vacuum manifold using sterile disposable forceps, gently folded in half and placed into a prelabeled 2.0-ml screw-cap microcentrifuge tube. For bead beating, the 2.0-ml tube contained 0.3 g of 1-mm zirconium silica beads (Biospec Corp., Bartlesville, OK). DNA was recovered from the organisms retained on the filters by addition of 600 µl of Buffer AE (QIAGEN, Valencia, CA). Approximately 10^5 *Lactococcus lactis* cells were added as SPC to each tube. Tubes were placed in a 48-position mini beadbeater (BioSpec Corp.) and shaken for 2 minutes at the highest speed setting. The tubes were then centrifuged at 12,000 x *g* for 1 minute to pellet the beads and debris. Resulting supernatants were transferred to sterile 1.6-ml microcentrifuge tubes. Sample processing using this method took less than 30 minutes for each sample. Full DNA extraction was conducted using the Mo Bio Fecal DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA)according to the manufacturer's instructions for maximum yield. Filters were processed using Mo Bio bead tubes to bead-beat for two minutes as described above.

Standards and Standard Curves

Standards for use in the QPCR assays were created based on cultures obtained from the American Type Culture Collection (ATCC) and cultured overnight at 37°C. *E. coli* (ATCC 25922) was grown in Tryptic Soy Broth, while *E. faecalis* (ATCC 29212) and *L. lactis* (ATCC 11454) were grown in Brain Heart Infusion Broth. Overnight cell suspensions were either counted fluorometrically (Noble and Fuhrman 1998) or by DST. Cell suspensions were diluted in PBS to concentrations listed below and frozen at -80°C in single-use aliquots. Aliquots were thawed and extracted in the same manner as described previously for samples.

Standard curves, produced by diluting the cell suspensions listed above, were processed in duplicate four-log dilutions for each reaction. Standard-curve cell counts ranged from 1.63 x 10^4 to 1.63 per reaction for *E. Faecalis*, and from 4.6 x 10^4 to 4.6 for *E. coli*. Inhibition was judged via the variation of 1/2 log (1.5 cycle threshold (Ct)) from the expected Ct of 26.58 for a spike of 1.0 x 10^5 SPC *L. lactis* cells that were co-extracted with the samples (i.e., if the Ct value for the sample was greater than 28.23 the sample was treated as inhibited). Inhibited samples were diluted 10-fold with sterile water and re-analyzed. Quantification during the first study (for which QPCR analysis was conducted by the method developers) relied on interpolation of cell numbers from the standard curve generated during each analysis. Results for the second study (for which QPCR analysis was conducted by OCSD microbiologists) were generated using the delta Ct approach (Haugland *et al*. 2005). Given the similarities observed between results calculated using standard curve and delta Ct approaches and results calculated by the EPA using the delta Ct approach, the delta Ct approach was adopted for the second part of this study. A calibrator curve was run in duplicate during each run using the calibrator (at a concentration of 1 x 10^5 *E. faecalis* cells) and three serial 10-fold dilutions. In this way, the amplification efficiency (*E*) was calculated using the given slope from the SmartCycler™ software (Cepheid): $E = 10^{(-slope)}$. The ratio of target DNA in the samples to that in the calibrator was calculated following Pfaffl (2001). The ratio was then multiplied by the amount of target DNA in the calibrator to get the sample quantities in number of cells.

QPCR Reactions

Lyophilized OmniMix® (Cepheid) and Total *Enterococcus*, *E. coli*, or *Lactococcus* SmartBeads were dissolved in RNase and DNase-free sterile water to create a master mix. For each master mix, 20-µl aliquots were pipetted into reaction tubes, followed by 5 µl of sample processed using bead beating only, bead beating followed by a commercial DNA extraction kit, or PBS for no template controls. The Omnimix and SmartBeads contained all required QPCR reagents, and primer/probe sets for *Enterococcus*, *E. coli*, or *Lactococcus*. All SmartBeads contained a propriety PCR positive internal control template (IC; Cepheid) and a primer/probe set (Cepheid) for this template. All

probes incorporated Scorpion chemistry. All reactions were prepared in 25-µl optical tubes (Cepheid). The reactions were monitored in a Smart Cycler II™ sequence detection instrument (Cepheid). Thermal cycling conditions for all reactions (*Enterococcus*/IC, *E. coli*/IC, and *Lactococcus*/IC) were the same, consisting of 1 cycle at 94°C for 2 minutes (hot start), then 45 cycles at 94°C for 5 seconds, and 62°C for 43 seconds (optics on). Determinations of Ct were performed automatically by the instrument after manually adjusting the threshold fluorescence value to 8 units. The Smartcycler II detected fluorescence emissions at three wavelengths that were specific to the fluorophores associated with each of the three probes. *Enterococcus* and *E. coli* probes were tagged with the fluorophore FAM (emission maximum at 515 nm), *Lactococcus* probes were tagged with the fluorophore Cy 5 (emission maximum at 554 nm), and the IC probes were tagged with the fluorophore Cal Fluor Red® (emission maximum at 610 nm; Biosearch Technologies, Inc., Novato, CA). Results of unknowns were calculated using fluorescence signals emitted at the correct wavelength and appropriate SmartCycler software associated with the respective standard curve generated or delta Ct.

During the second study conducted by OCSD personnel, samples were processed as described above, but results are only presented for the bead beating approach. At the beginning of the second study, samples were processed using both bead beating only and bead beating plus a commercial DNA extraction kit. After processing of roughly 40 samples in the second study, we determined that the use of a full commercial DNA extraction kit proved to be too difficult and time consuming for OCSD microbiologists, and that bead beating only fit better into the work flow of their daily routine monitoring. Therefore, for the remainder of the second study the samples were processed using only bead beating. Through examination of the first and second study data, we also observed that the standard curve and delta Ct quantification approaches yielded highly similar results. Therefore, during the second study, to reduce resource expenditure the delta Ct quantification approach was employed as by Haugland *et al*. (2005). The delta Ct method uses an abridged standard curve of the respective calibrator, *Enterococcus* sp. or *E. coli*, to derive the QPCR amplification efficiency (E_{QPCR}). The ratio of change between the calibrator Ct for a known cell amount and the unknown Ct was multiplied by E_{QPCR} to arrive at the cell number. Even though the delta Ct approach was used for enumeration, standard curves were run with every sample batch to assess *E*, which exceeded 90% for every run.

Data Handling and Statistical Calculations

All QPCR- and culture-based datasets were tested for normality and failed. Data was log-transformed and subsequent *Enterococcus* or *E. coli* QPCR results were compared to the culture-based method results using Pearson product-moment analysis. For *E. coli*, we compared QPCR cell equivalent results to mean Colilert-18 analyses only. Non-detect culture-based values were reported as one-half of the detection limit (i.e., <10 became 5) and greater-than values were deleted from the data pool. Samples that yielded a non-detect QPCR result were assigned a concentration of 5 cells per 100 ml. To assess inhibition, the results for the SPC (*Lactococcus*) and the IC for each reaction were examined. Inhibition was defined as a delay in amplification by 1.5 Ct of an unknown as compared to either a filter processed only with the SPC, or the IC with no added sample. Although samples were identifies as inhibited, they were sitll included in the overall analyses for *E. coli*.

Results

Testing Conducted by Method Developer

Concentrations of *Enterococcus* sp. measured using QPCR were significantly correlated with those as measured using Enterolert and EPA Method 1600 (Table 2; Figure 1). However, the correlation with Enterolert was stronger for samples processed using bead beating alone. These correlation coefficients compared favorably with, but were not quite as strong as, the relationship between the two culture-based methods.

The slope of the regression for QPCR-based vs. culture-based methods was less than 1.0, indicating relative underestimation by QPCR. The slope was higher for samples processed using bead beating only, suggesting some loss of target cells in the DNA extraction step. The correlation between *E. coli* QPCR and Colilert-18 was also significant and of nearly the same magnitude as for *Enterococcus* QPCR (Table 2; Figure 2). However, unlike *Enterococcus* QPCR, the slope of the relationship for culture-based methods was nearly unity for both bead beating and bead beating plus the commercial

Table 2. Regression analysis results for the first and second testing of rapid QPCR methods for *Enterococcus* and *E. coli* as compared to culture-based methods. First study testing conducted by method developers; second study testing conducted by water quality microbiologists. BB = bead beating; BB+DNA = bead beating plus DNA purification using a commercial extraction kit.

	Method	Best Linear Fit	n	R	p-value
First Study					
Enterococcus	EPA Method 1600 vs. QPCR BB	y=0.8023x +0.6879	54	0.79	<0.0001
	EPA Method 1600 vs. QPCR BB+DNA	y=0.7379x +0.8038	54	0.78	<0.0001
	Enterolert vs. QPCR BB	y=0.8653x +0.3806	54	0.84	<0.0001
	Enterolert vs. QPCR BB+DNA	y=0.7513x +0.6361	54	0.79	<0.0001
	EPA Method 1600 vs. Enterolert	y=0.9254x +0.3593	54	0.94	<0.0001
E. coli	Colilert-18 vs. QPCR BB	y=1.0346x+0.0324	53	0.86	<0.001
E. coli	Colilert-18 vs. QPCR BB+DNA	y= 0.9635x +0.0991	54	0.83	<0.0001
Second Study					
Enterococcus	EPA Method 1600 vs. QPCR	y=0.7509x + 0.1559	146	0.86	<0.0001
	Enterolert vs. QPCR	y =0.7516x +0.2549	144	0.83	<0.0001
	EPA Method 1600 vs. Enterolert	y=0.8869x + 0.0942	152	0.91	<0.0001
E. coli	Colilert-18 vs. QPCR	y=0.7058x + 0.0596	119	0.84	<0.001

DNA extraction kit: y = 1.0346x + 0.0324 and y = 0.9635x + 0.0991, respectively.

Repeatability between duplicates was similar between QPCR and culture-based methods for *Enterococcus* sp. (Table 3). The QPCR methods using bead beating yielded a slightly smaller coefficient of variation (CoV) than that for EPA Method 1600 and Enterolert, and even smaller CoV for samples processed using bead beating followed by DNA extraction. In contrast, culture-based methods had substantially lower CoV than the CoV for QPCR-based methods for *E. coli* (Table 3).

Testing Conducted by Water Quality Microbiologists

The correlation between QPCR- and culture-based methods for *Enterococcus* was nearly the same when employed by the OCSD microbiology laboratory personnel as when employed by the method developers (Table 2; Figures 3 and 4). Moreover, the correlation between QPCR and Enterolert and EPA Method 1600 was nearly as high as that between the two culture-based methods (Table 2). When the results were examined with respect to whether the measurement exceeded 104 colony forming units (CFU) per 100 ml, the concentration at which beach water quality warnings are issued, the *Enterococcus* QPCR agreed with EPA Method 1600 and Enterolert for 88% and 87% of the samples, respectively. This was close to the 94% agreement rate between the two culture-based methods.

The relationship between QPCR and Colilert-18 was even stronger for *E. coli* than that observed during the testing conducted by the method developers (Table 2; Figures 2 and 4). When assessed relative to the beach warning decision criterion, the agreement rate between QPCR and Colilert-18 was 94%.

Repeatability of the *Enterococcus* QPCR assay was nearly the same as that for the culture-based methods when testing was conducted by water quality agency personnel (Table 3). Unlike the testing conducted by the method developers, for which we

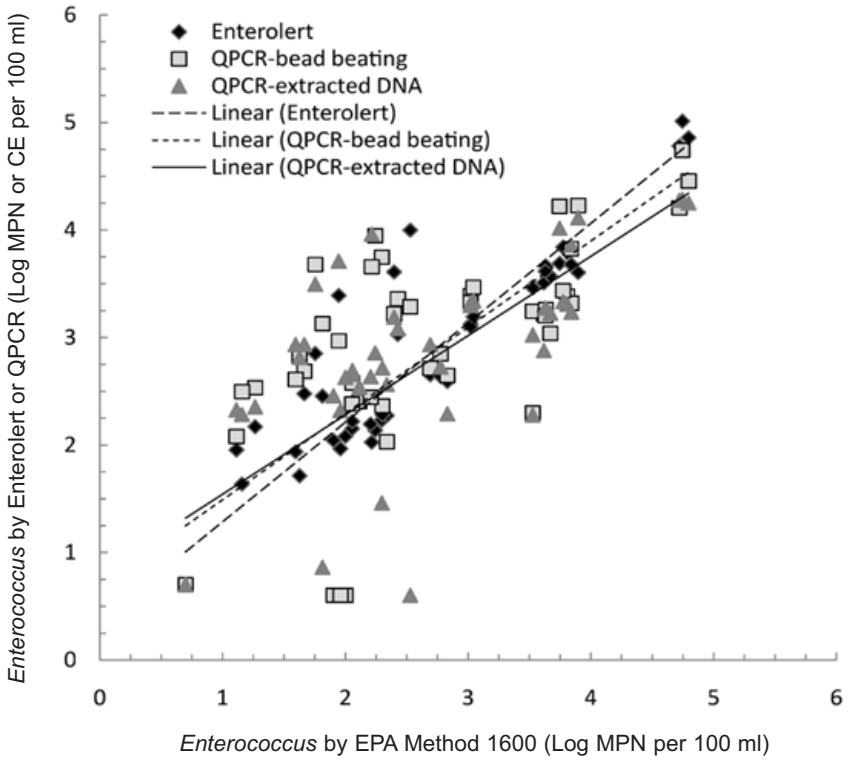

Figure 1. Comparison among multiple measures of *Enterococcus* sp. concentration: EPA Method 1600 versus Enterolert (black diamonds) or *Enterococcus* QPCR for a range of water samples. QPCR testing was conducted by the method developers. Log-transformed EPA Method 1600 versus log-transformed rapid QPCR for *Enterococcus* results (gray squares) represent samples processed using bead beating only; gray triangles represent samples processed using bead beating followed by a commercial DNA extraction kit. CE = cell equivalents; MPN = most probable number. Best linear fit equations are reported in Table 2.

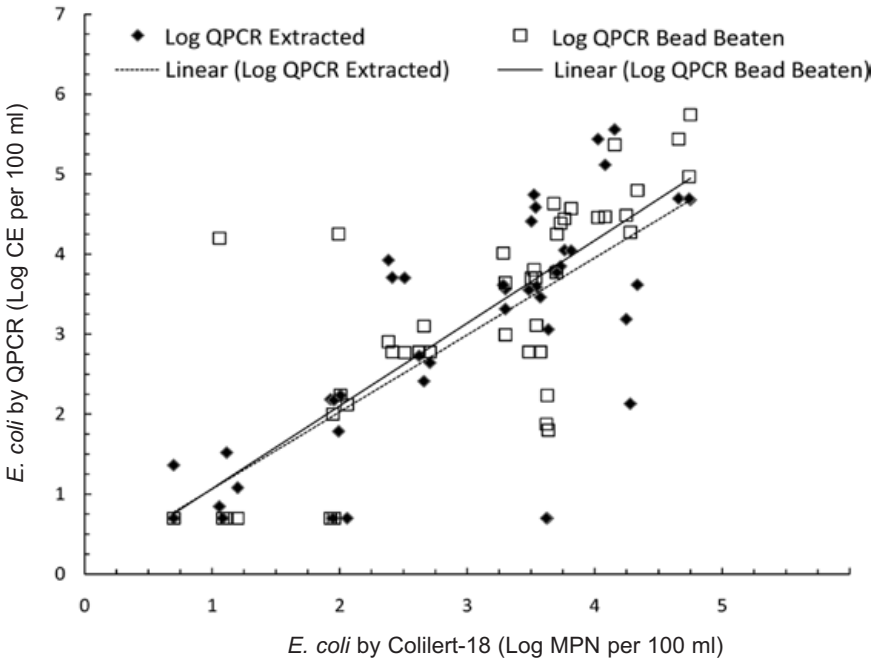

Figure 2. Comparison between Colilert-18 and QPCR for a range of water samples. The *E. coli* QPCR testing was conducted by the method developers. Log-transformed Colilert-18 results represent samples processed using bead beating only; log-transformed QPCR results represent samples processed using bead beating followed by a commercial DNA extraction kit. CE = cell equivalents; MPN = most probable number.

Table 3. Average coefficient of variation for testing methods for enumeration of *Enterococcus* sp. and *E. coli* during the two studies. BB = bead beating.

Testing Conducted by:		Coefficient of Variation
Method Developers		
Enterococcus	QPCR: BB	0.27
	QPCR: BB + DNA	0.36
	Enterolert	0.31
	EPA Method 1600	0.32
E. coli	QPCR BB only	0.67
	Colilert-18	0.19
Water Quality Microbiologists		
Enterococcus	QPCR: BB	0.21
	Enterolert	0.21
	EPA Method 1600	0.18
E. coli	QPCR: BB	0.25
	Colilert-18	0.25

observed a large difference in CoV between the *E. coli* QPCR assay and Colilert-18, the CoV for the two methods during the testing conducted by the water quality microbiologists was identical.

Identified as delay in the SPC and IC amplification of more than 1.5 Ct values, inhibition was observed in 8 of the 163 samples. Three of these were enclosed beach samples, and five were storm drain samples. The three enclosed beach samples were collected from Newport Dunes, in Newport Beach CA. Four of the inhibited storm drain samples were collected from the same location: Back Bay Storm Drain in Newport Beach, CA.

Discussion

Results based on QPCR-based methods were significantly correlated with, and as repeatable as, EPA approved culture-based methods; however, the slope of the correlation indicates a bias toward underestimation by QPCR relative to culture-based methods. This contrasts with concerns that have been expressed about potential QPCR overestimation relative to culture-based methods because it does not differentiate DNA fragments from culturable cells. Notably, we did not observe such overestimation in samples analyzed during this study, even when examining the sewage influent or effluent samples alone (data not shown).

There are several possible explanations for the observed underestimation; one of which is inhibition of DNA amplification during QPCR. Inhibition typically occurs when high molecular weight compounds, and heavy metals in the source water (e.g., humic acids and other complex carbohydrates) combine with metal ions to sequester nucleic acids from polymerases and prevent amplification (Thurman *et al*. 1988, Tsai and Olson 1992, De Boer *et al*. 1995, Kreader 1996, Watson and Blackwell 2000). We observed a lower slope for storm drain samples than for the beach samples, which is consistent with inhibition because storm drain samples contain a complex mixture of organic inputs running off of the urbanized landscape. Notably, we did not observe the same extent of inhibition for the *E. coli* QPCR analysis. Inhibition should be detected by the SPC and IC which were incorporated into the analysis. Although inhibition was observed in five of the storm drain samples analyzed for *Enteroccocus* sp., it is possible that the criteria used for the SPC and IC may have been too lenient resulting in missed identification of inhibited samples. However, inhibited samples were included in the *E. coli* comparison because the QPCR analysis produced Ct values; removing inhibited samples would have resulted in even closer agreement betweeen QPCR- and culture-based results.

Another possible explanation for underestimation is that the molecular primers may be more specific to the target species, as compared to the wide range of *Enterococcus* species that are enumerated using EPA Method 1600. We used a pan-*Enterococcus* primer-probe set for *Enterococcus* QPCR in an attempt to detect a wide range of members of the *Enterococcus* genus. However, this approach may have been too specific to include all *Enterococcus* species that grow on the mEI agar used for EPA Method 1600. For example, EPA Method 1600 has been reported to grow a range of non-target species, with false positives rates as high as 17 - 40% reported in some instances (Moore *et al*. 2008).

Regardless of the reason, underestimation is a substantial management concern because beach managers place high priority on ensuring that the public is not swimming in contaminated water. A false negative, in which a sample that actually exceeds standards is measured as below standards, is problematic because there is no subsequent mechanism for deter-

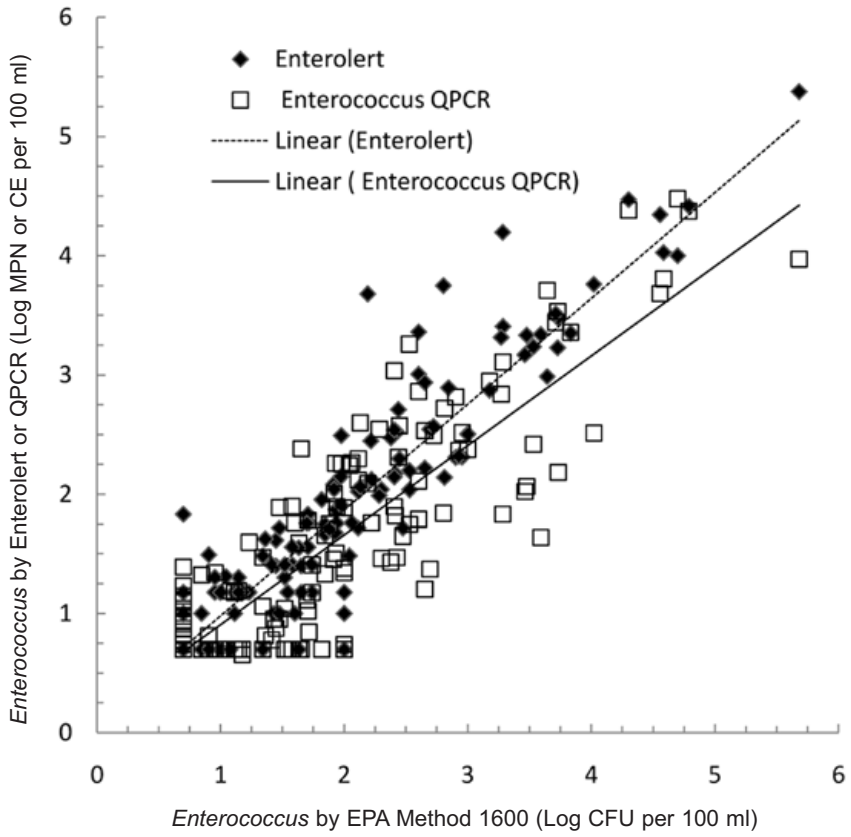

Figure 3. Comparison among multiple measures of *Enterococcus* sp. concentration: EPA Method 1600 versus Enterolert or *Enterococcus* QPCR for a range of ambient southern California marine water samples. The QPCR testing was conducted by water quality personnel at Orange County Sanitation District. All QPCR analyses were conducted on samples processed using bead beating only. CFU = colony forming units; CE = cell equivalents; MPN = most probable number. Best linear fit equations are reported in Table 2.

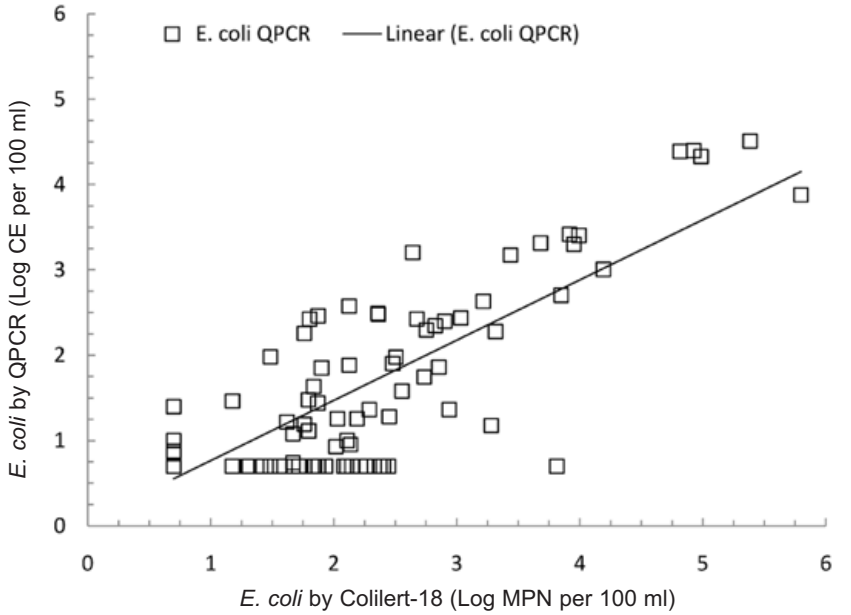

Figure 4. Comparison between concentrations of *E. coli* measured using Colilert-18 and *E. coli* QPCR for a range of ambient southern California marine water samples. The QPCR testing was conducted by water quality personnel at Orange County Sanitation District. All QPCR analyses were conducted on samples processed using bead beating only. CE = cell equivalents; MPN = most probable number. Best linear fit equations are reported in Table 2.

mining that a problem exists. In contrast, a false positive would lead to an inappropriate warning, but one which could be remedied by additional sampling with alternative methods that would be triggered by the positive measurement.

The second study permitted assessment of whether QPCR technology could be successfully transferred to a local laboratory, which we generally found to be the case. The OCSD microbiology personnel were able to produce results in about two hours, even though their testing included a wider array of sample types than in the first study. The relationships with EPA approved methods and repeatability between replicates were as strong as those observed for QPCR assays conducted by the method developers. However, not all aspects of technology transfer were successful. We abandoned DNA extraction prior to QPCR, which was intended to reduce inhibition, was abandoned quickly because OCSD staff found it complex and time consuming. In particular, they found that the many pipeting steps introduced opportunities for imprecision, which was confirmed by the observed CoV between replicates being three times higher than that for samples processed using bead beating. In contrast, the lyophilized bead technology associated with sample processing that used beat beating only reduced sample manipulation to only two pipeting steps. Additional method automation is desirable, particularly if more complex procedures to minimize inhibition are to be adopted by similar water quality monitoring laboratories across the country.

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