Correlation between quantitative polymerase chain reaction and culture-based methods for measuring *Enterococcus* over various temporal scales and three California marine beaches

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Abstract

Several studies have examined how fecal indicator bacteria (FIB) measurements compare between quantitative polymerase chain reaction (QPCR) and the culture methods it is intended to replace. Here we extend those studies by examining the stability of that relationship within a beach, as affected by time of day and seasonal variations in source. *Enterococcus* spp. were quantified at three southern California beaches in the morning and afternoon using two QPCR assays, membrane filtration, and defined-substrate testing. While QPCR and culture-based measurements were consistently and significantly correlated, strength of the correlation varied both among and within beaches. Correlations were higher in the morning ($0.45< \rho <0.74$) than in the afternoon ($0.18< \rho <0.45$), and higher when the fecal contamination was concentrated ($0.38< \rho <0.83$) than when it was diffuse ($0.19< \rho <0.34$). The ratios between culture-based and QPCR results (colony forming units (CFU) or most probable number (MPN) per calibrator cell equivalents (CCE)) also varied spatially and temporally. Ratios ranged between 0.04 and 0.85 CFU or MPN per CCE, and were lowest at the beach affected by diffuse pollution. Patterns in the ratios over the course of the day were dissimilar across beaches, increasing with time at one beach and decreasing at another. The spatial and temporal variability we observed indicate that the empirical relationship between culture-based and QPCR results is not universal, even within a beach.

Introduction

Recreational beach water quality has been assessed using culture-based measurements of fecal indicator bacteria (FIB) for nearly a century. These methods have achieved widespread usage because they are cost effective, easily implemented and correlate well with health risk (Wade *et al.* 2003). However, culture-based methods are slow, requiring 18 to 96 hours from sample collection to results, a timeframe inappropriate for monitoring beach contamination that is often episodic and of short

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duration (Leecaster and Weisberg 2001; Boehm *et al.* 2002, 2009).

Quantitative polymerase chain reaction (QPCR) is a new method that eliminates the incubation step by directly measuring genetic material and thereby reducing measurement time to as little as two hours (Noble and Weisberg 2005). QPCR-based measurements of FIB have been found to relate well to health risk (Wade *et al.* 2006, 2008, 2010) and the increased speed provides substantial advantage in health protection. Early applications of QPCR have been successful, but cost and logistical challenges will likely limit application of QPCR to a subset of beaches (Griffith and Weisberg 2011).

As both QPCR and culture-based methods will likely be used for beach monitoring in the future, there is a need to understand how often and under what circumstances they yield different results. QPCR does not necessarily produce results comparable to culture-based methods because QPCR measures a genetic, rather than a growth, endpoint. Several studies have found correlations between QPCR and culture-based methods (e.g., Lavender and Kinzelman 2009, Noble *et al.* 2010, Whitman *et al.* 2010), though QPCR has been found to produce higher values under some circumstances (He and Jiang 2005, Haugland *et al.* 2005, Morrison *et al.* 2008, Lavender and Kinzelman 2009, Abdelzaher *et al.* 2010, Griffith and Weisberg 2011). These comparative studies have generally been spatially extensive, based upon a small number of samples collected from a large number of beaches. It remains unclear whether the linear relationship between culture-based and QPCR results, represented by the ratio of culture to QPCR results, is constant or predictable over time. Here we extend the previous studies by collecting a large number of samples from three beaches to assess the stability of the relationship between enumeration method results over time of day and season.

Methods

Water samples were collected from three southern California beaches: Avalon Bay Beach, Catalina Island; Doheny State Beach, Dana Point; and Surfrider Beach, Malibu. At Avalon, samples were collected at four beach locations at 8:00 a.m., 12:00 p.m., and 3:00 p.m. for 27 days and 31 days between May and September in 2007 and 2008, respectively. At Doheny, five beach locations were sampled at 8:00 a.m., 1:00 p.m., and 3:00 p.m. for 8 days in May through July 2007 and 31 days between May and September 2008. At Surfrider, five beach locations were sampled at 8:00 a.m. and 1:00 p.m. for 39 days from May to September in 2009. Surface water discharges are sometimes affected seasonally by naturally occurring sand berms that restrict flow to the ocean at Doheny and Surfrider. These berms were present for all but eight sampling days at Doheny and five at Surfrider.

At all beaches, samples were collected at approximately 0.5 m depth in five-gallon buckets that were sterilized with 10% bleach and rinsed with 1% sodium thiosulfate. Samples were immediately mixed in 50-gallon tanks using 0.7 m x 0.7 m stir plates and 20 cm stir bars. After 15 minutes of stirring, subsamples (approximately 2 L) were decanted into sterile 4 L cubitainers. From these subsamples, 100 or 200 ml were filtered onto 47 mm, 0.4 μm pore size polycarbonate filters (HTTP; Millipore Corp., Bedford, MA) and flash frozen in liquid nitrogen for future QPCR analyses. Two hundred milliliters were filtered at Avalon in 2008; 100 ml were filtered at the other two beaches and at Avalon in 2007.

Culture-based Analyses of Enterococcus spp. Concentrations

Enterococcus spp. were enumerated by membrane filtration (MF) following EPA Method 1600 (APHA 2005). Concentrations were also measured using Enterolert® (IDEXX Laboratories, Westbrook, ME) following manufacturer's instructions and using the manufacturer-provided Most Probable Number (MPN) table.

DNA Recovery for QPCR:

Frozen polycarbonate filters were transferred to 2 ml semiconical screw-cap microcentrifuge tubes pre-loaded with 0.3 g of 0.1 mm zirconia/silica beads (Biospec Corp., Bartlesville, OK). Five hundred or six-hundred microliters of AE buffer (QIAGEN, Valencia, CA) with 0.2 μg/ml of salmon testes DNA (Sigma, St. Louis, MO) were added to each sample, calibrator, and negative control. The salmon testes DNA served as a specimen processing control (SPC), used to estimate sample loss during DNA recovery and to identify presumptive PCR inhibition. Tubes were then bead-milled in an eight-position mini bead beater (Biospec Corp.) for two min, followed by centrifugation for 1 min at 12,000 x g. Supernatants

were transferred to 1.7 ml microcentrifuge tubes and centrifuged at 12,000 x g for 5 minutes Supernatant was transferred to a sterile 1.7 ml microcentrifuge tube, stored at 4°C, and QPCR-analyzed within one week.

QPCR Analyses

Two *Enterococcus* spp. QPCR analyses were conducted on DNA recovered from replicate filters. Both assays targeted the multiple copy 23S rRNA gene in an approach similar to that outlined by Ludwig and Schleifer (2000). The first used TaqMan chemistry was described by Haugland *et al.* (2005). The second was based on Scorpion® chemistry, generally following the procedure outlined by Noble *et al.* (2010).

The Scorpion® QPCR assays (hereafter referred to as EntScorp) were conducted as 25 µl reactions using OmniMix beads (a lyophilized premix with 1.5 units of TaKaRa hot start Taq polymerase, 200 µM dNTPs, 4 mM $MgCl_2$, and 25 mM HEPES with a pH of 8; Cepheid), 0.25 µM of the forward primer, 0.25 µM of the probe, and 5 µl of the sample DNA. Reactions were thermal cycled and monitored in a SmartCycler II® (Cepheid). Thermal cycling occurred in two stages: first, 2 min at 95°C, followed by 45 cycles of 5 s at 94°C and 43 s at 62°C. The 25-microliter SPC reactions were prepared with OmniMix, 1.0 µM of each primer, 0.1 µM of the TaqMan probe and 5 µl of sample. These reactions were thermal cycled at 95°C for 2 minutes, followed by 45 cycles of 15 s at 94°C and 30 s at 60°C.

The TaqMan *Enterococcus* spp. QPCR assays (hereafter referred to as EntTaq) were conducted following Haugland *et al.* (2005). Briefly, 25 µl reactions were prepared with 12.5 µl of TaqMan™ Universal Master Mix (Applied Biosystems), 5 µM each of the forward and reverse primers, 400 nM probe, 2.5 µl of 2 mg/ml bovine serum albumin, and 5 µl of sample DNA diluted 1:10 in water. Cycling conditions consisted of 2 minutes at 50°C, 10 minutes at 95°C, followed by 45 cycles of 15 s at 95°C and 1 minute at 60°C. The SPC assay was conducted in 25 µL reactions, using 12.5 µl of TaqMan Universal Master Mix, 5 µM of each primer, 400 nM of probe, 2.5 µl of 2 mg/ml bovine serum albumin, and 5 µl of sample diluted 1:10. Thermal cycling conditions were the same as those for the EntTaq assay.

Enterococcus faecalis (American Type Culture Collection 29212) cells were used to create QPCR calibrators. Cell lines were obtained from the ATCC and cultured overnight at 37°C in brain heart infusion broth. Cells were counted spectrophotometrically after 18 hours, and cell suspensions were diluted with phosphate buffered saline (PBS). Calibration standards were prepared by filtering 100,000 cells onto 47 mm, 0.4 µm pore size polycarbonate filters. Filters were stored between -70°C and -80°C until sample DNA recovery for QPCR analyses. A four-point duplicate standard curve was run during each reaction using the calibrator and three serial 10-fold dilutions. Amplification efficiency was calculated using the slope of the log standard curve: E=10^(-slope).

For both assays, presumptive sample inhibition and extraction loss were estimated using a TaqMan-based QPCR assay targeting the SPC (Haugland *et al.* 2005). For the EntTaq assay, cell concentrations were calculated using the ΔΔCt method described in Haugland *et al.* (2005), which is based on the relative quantity of target DNA in a sample compared to that in a known quantity of target organisms (the calibrator). Results were normalized for DNA recovery by comparing the recovered quantities of the SPC in each sample to the amount of SPC in the calibrator. Samples with more than a 3 Ct delay in the SPC were considered inhibited, diluted 1:5 in sterile water, and reanalyzed.

The EntScorp assay results were quantified using the ΔCt method outlined in Pfaffl (2001) with adjustments for the amplification efficiency. Like the ΔΔCt method, the ratio of the sample Ct value to the calibrator Ct value was multiplied by the amount of target cells in the calibrator to quantify the total number of calibrator cell equivalents (CCE), but the SPC Cts were not used to quantitatively modify results for DNA recovery. Samples with more than a 1.6 Ct delay in the SPC were diluted with sterile water and reanalyzed.

Data Analyses

The ratios between *Enterococcus* spp. concentrations among methods were compared: 1) among the three study beaches; 2) between open- and closed-berm days at Doheny and Surfrider beaches; and 3) among morning and afternoon samples. Ratios were calculated by dividing the culture result by the QPCR result for each sample. Wilcoxon Signed Rank, Kruskal-Wallis, and Friedman tests were used to determine whether differences in the ratios were significant, and Spearman correlations

were calculated between method results. When multiple pair-wise comparisons were made on a single data set, the significance level was adjusted following Bonferroni.

All concentrations were normalized to CE, MPN, or CFU per 100 ml. Samples yielding a QPCR non-detect or a measurable concentration below the MF detection limits were assigned a value of 2 calibrator cell equivalents (CCE) or colony-forming units (CFU) per 100 ml. Samples yielding an Enterolert non-detect were assigned a value of 10 MPN per 100 ml because all samples were run at a dilution of 1:10. Because method variability is greater at low concentrations (Whitman *et al.* 2010), analysis was conducted twice, once using the full data set and once removing samples with concentrations less than 80 MPN, CCE, or CFU per 100 ml to elicit relationships at the higher concentrations.

Results

Concentrations of *Enterococcus* spp. varied by method and beach (Figure 1). Average *Enterococcus* spp. concentrations as measured by Enterolert were similar among the three study beaches hovering near $10^{1.5}$ MPN per 100 ml. Average MF-measured *Enterococcus* spp. concentrations were similar at Avalon and Doheny ($10^{1.5}$ CFU per 100 ml) but were lower at Surfrider (10 CFU per 100 ml). The EntTaq and EntScorp assays generated similar results regardless of beach. Both QPCR methods had significantly higher average concentrations ($10^{2.3}$ CCE per 100 ml) at Avalon than either Doheny or Surfrider ($10^{1.5}$ CCE per 100 ml).

When results were examined with respect to a single sample standard of 104 CFU, MPN or CCE per 100 ml, QPCR results agreed with culture results in 76 to 85% of samples at Doheny and Surfrider Beaches (Table 1). Agreement was considerably

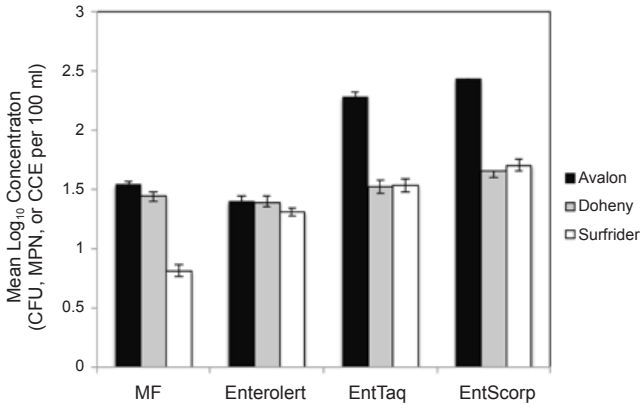

Figure 1. Mean log10 concentration of *Enterococcus* spp. as measured by membrane filtration, Enterolert, and the QPCR assays at each beach. Error bars indicate standard error.

worse at Avalon, where QPCR and culture results would yield the same management decision only 31 to 43% of the time. These values showed 96 to 98% agreement between the two culture-based methods at Surfrider and Doheny and 80% at Avalon. In most cases of disagreement, QPCR results suggested beach closure while culture results would leave the beach open. It is important to bear in mind, though, that this comparison is based upon the arbitrary assignment of the culture method standard to QPCR results. QPCR standards will need to be established from epidemiological work and may not coincide with culture standards.

Ratios between culture and QPCR results also differed between beaches (Figure 2a), being highest at Doheny and lowest at Avalon. When data were truncated, the ratio was lower at Surfrider and Doheny, sometimes by an order of magnitude (Figure 2b). Ratios were consistent at Avalon using the entire or truncated data set.

Table 1. Percent of the time that management decisions based upon QPCR agree with those based upon culture methods when using the single sample standard of 104 CFU, MPN, or CE per 100 ml. Sample size (N) is given in parentheses.

Site	MF vs Enterolert	Enterolert vs EntTaq	Enterolert vs EntScorp	MF vs EntTaq	MF vs EntScorp
Surfrider	97.6% (333)	85.0% (333)	75.7% (333)	84.7% (333)	76.3% (333)
Doheny	95.9% (337)	85.2% (337)	81.3% (337)	85.8% (337)	83.1% (337)
Avalon	80.6% (530)	32.9% (529)	31.5% (530)	43.1% (615)	41.9% (616)

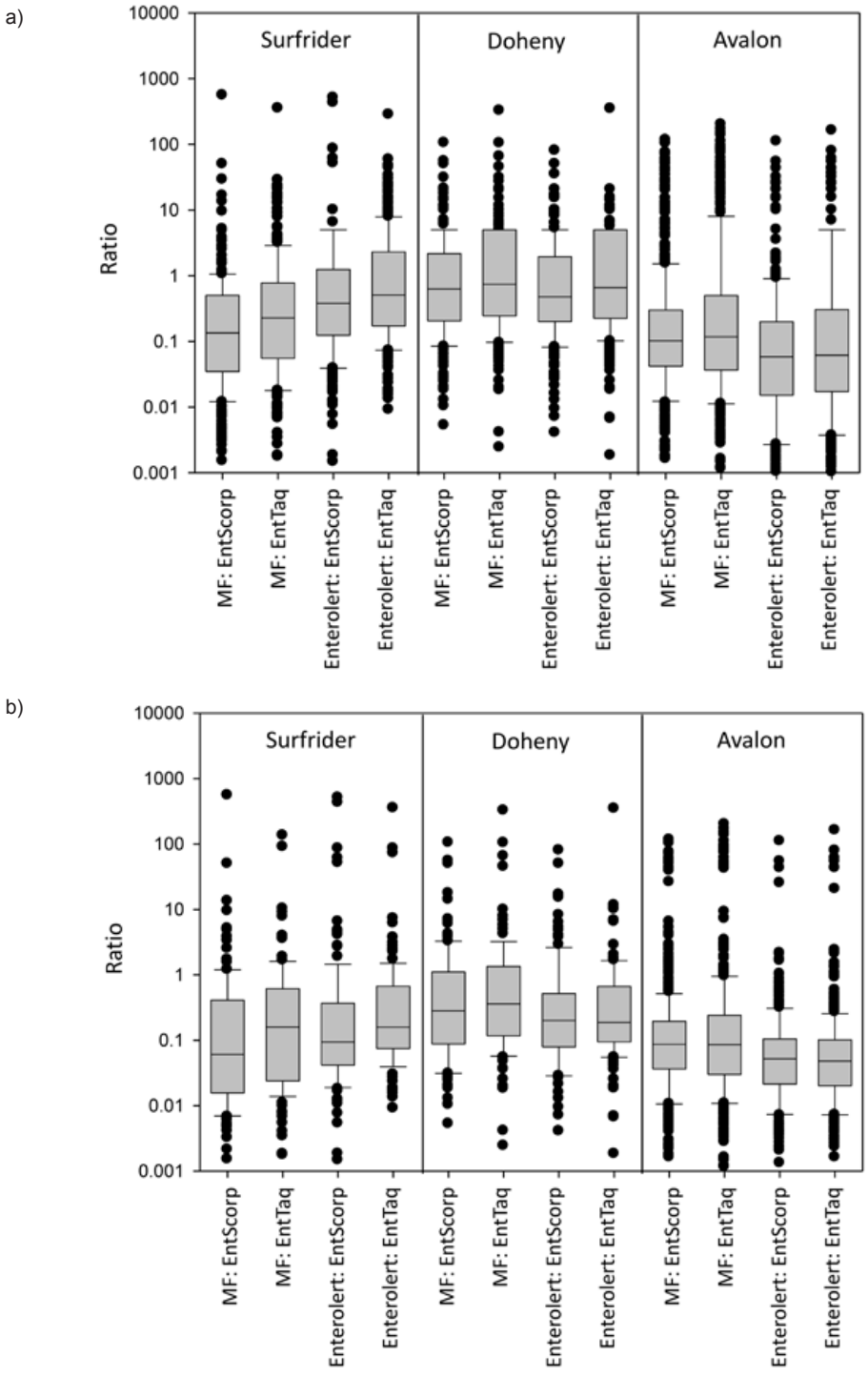

Figure 2. Ratio of culture-based results to QPCR results at each beach using the entire dataset (a) and the truncated dataset (b).

Comparison across Time of Day

Concentrations measured by all methods were significantly greater in the morning than later in the day at all sites (Supplemental Information (SI) Figure SI-1). When data were examined by time of day, *Enterococcus* spp. concentrations as measured by QPCR and Enterolert were higher in the morning than in the afternoon by a factor of five at Avalon and Doheny. MF-measured concentrations were an order of magnitude higher in the morning.

Ratios between method results were compared among morning and afternoon samples at Doheny and Avalon (Table 2). Surfrider was excluded from this analysis because no afternoon samples were

Table 2. Median ratios between culture and QPCR results (culture result/QPCR result). Bolded values indicate a significant difference in ratios across time of day. Sample size (N) is given in parentheses.

Site	Method	All Data						Truncated Data					
		MF			Enterolert			MF			Enterolert		
		AM	Mid	PM	AM	Mid	PM	AM	Mid	PM	AM	Mid	PM
Avalon	EntTaq	**0.11** (201)	**0.12** (246)	**0.15** (170)	0.07 (201)	0.07 (172)	0.08 (164)	**0.08** (165)	**0.07** (198)	**0.1** (130)	0.04 (156)	0.05 (137)	0.05 (116)
	EntScorp	**0.09** (201)	**0.09** (246)	**0.16** (170)	0.07 (201)	0.07 (173)	0.08 (164)	**0.08** (165)	**0.08** (198)	**0.12** (130)	0.05 (165)	0.04 (135)	0.07 (130)
Doheny	EntTaq	0.76 (130)	0.8 (104)	0.7 (103)	0.57 (130)	0.59 (104)	0.73 (103)	0.42 (64)	0.36 (25)	0.21 (29)	0.25 (61)	0.12 (23)	0.12 (27)
	EntScorp	0.58 (130)	0.47 (104)	0.41 (103)	0.58 (130)	0.47 (104)	0.41 (103)	**0.57** (67)	**0.1** (30)	**0.41** (29)	**0.31** (67)	**0.09** (30)	**0.1** (28)

collected. At Avalon, the ratio tended to increase, often significantly, as the day progressed (i.e., the QPCR results declined relatively faster than the culture results). At Doheny, the ratio was often lower in the afternoon than in the morning, though differences were rarely significant. The ratio trends were similar when the data set was truncated.

Correlations between culture and QPCR methods weakened over the course of the day at every beach (Table 3). At Doheny and Surfrider, ρ ranged from 0.59 to 0.74 in the morning and 0.16 to 0.51 in the mid-day and afternoon, respectively. Correlations were generally weaker at Avalon, but the same trend was observed: $0.45 < \rho < 0.53$ in the morning and $0.18 < \rho < 0.39$ in the afternoon.

Comparison across Berm Status

At both Doheny and Surfrider, the beach is hydrologically connected to urban runoff from upstream, but this flow is often interrupted in the

Table 3. Significant Spearman Rank correlations between log-transformed *Enterococcus* sp. concentrations as measured by various methods depending upon time of day.

Site	Time of Day	MF vs. Enterolert	EntTaq vs. Enterolert	EntScorp vs. Enterolert	EntTaq vs. MF	EntScorp vs. MF
Surfrider	AM	0.83	0.7	0.59	0.67	0.65
	Mid-day	0.82	not significant	0.16	0.25	0.31
Doheny	AM	0.85	0.74	0.74	0.69	0.71
	Mid-day	0.7	0.51	0.6	0.44	0.49
	PM	0.54	0.31	0.31	0.46	0.43
Avalon	AM	0.74	0.45	0.45	0.47	0.53
	Mid-day	0.55	not significant	0.28	0.18	0.45
	PM	0.57	0.18	0.2	0.18	0.39

summer when sand berms form at the beach under low flow conditions. Berms were in place on 75% of study days at Doheny and 65% at Surfrider. At both beaches, *Enterococcus* spp. concentrations were higher by a factor of five to ten by all methods when the berms were open (Figure SI-2). The relationships in method ratios between periods when the berm was open and when the berm was closed were different depending upon data inclusion. When all data were included, the ratios between culture and QPCR results tended to be higher when the berm was closed than when it was open (Table 4). The opposite was true when the truncated data set was used, with higher ratios when the berm was open in all but one case.

The correlations between culture and QPCR method results were stronger when the berm was open than when it was closed at both beaches (Table 5). At Doheny, correlation coefficients between culture and QPCR results ranged between 0.71 and 0.83 when the berm was open and 0.20 and 0.27 when the berm was closed. Correlations were weaker at Surfrider, but the same trend was observed: $0.38 < \rho < 0.60$ when the berm was open and $0.19 < \rho < 0.34$ when the berm was closed.

Inhibition

The frequency of samples that failed SPC assay tests for potential QPCR inhibition varied by method and in some instances across beaches. When Scorpion method results from all beaches were pooled, 16% of samples were above the 1.6 Ct acceptance threshold for this method and required further dilution. Separating the data by beach, failure rates at Avalon (including 100 and 200 ml samples), Doheny, and Surfrider were 22, 1, and 20%, respectively. In contrast, when TaqMan method results were pooled 7% of samples were above the 3 Ct threshold value. Failure rates were 13% at Avalon (including both 100 and 200 ml samples), 2% at Doheny, and 3% at Surfrider. Additional studies are needed to determine the ability of the SPC assay and the alternative acceptance threshold values to correctly identify significant QPCR inhibition as well as the comparative effects of inhibitors on the different methods and the accuracy of SPC assay based adjustments in recovery estimates from the ΔΔCT calculation method.

Discussion

QPCR consistently yielded higher *Enterococcus* spp. values than either culture method, likely reflecting that QPCR measures the presence of

Table 4. Median ratios between culture and QPCR results (culture result/QPCR result). Bolded values indicate a significant difference with berm status. Sample size (N) is given in parentheses.

Site	Method	All Data				Truncated Data			
		MF		Enterolert		MF		Enterolert	
		Open	Closed	Open	Closed	Open	Closed	Open	Closed
Doheny	EntTaq	**0.57**	**0.84**	**0.48**	**0.60**	0.57	0.21	**0.40**	**0.12**
		(48)	(143)	(48)	(143)	(30)	(32)	(30)	(27)
	EntScorp	**0.30**	**0.72**	**0.21**	**0.58**	0.28	0.36	0.18	0.10
		(48)	(143)	(48)	(143)	(41)	(34)	(41)	(23)
Surfrider	EntTaq	0.27	0.30	0.25	0.24	**0.14**	**0.03**	**0.12**	**0.07**
		(64)	(116)	(64)	(116)	(28)	(25)	(28)	(25)
	EntScorp	0.16	0.18	**0.24**	**0.45**	**0.11**	**0.02**	**0.08**	**0.05**
		(64)	(117)	(64)	(117)	(28)	(26)	(28)	(34)

Table 5. Significant Spearman Rank correlations between log-transformed *Enterococcus* sp. concentrations as measured by various methods depending upon berm status.

Site	Berm Status	MF vs. Enterolert	EntTaq vs. Enterolert	EntScorp vs. Enterolert	EntTaq vs. MF	EntScorp vs. MF
Doheny	Open	0.90	0.79	0.71	0.83	0.74
	Closed	0.55	0.20	0.24	0.27	0.25
Surfrider	Open	0.73	0.44	0.51	0.38	0.60
	Closed	0.42	0.25	0.19	0.23	0.34

genetic material while the culture methods measure viable cells. Previous studies have found improved correlations between enumeration methods when fecal contamination is fresh and delivered in a concentrated pulse (Noble *et al.* 2010) probably because there is reduced time for decoupling of cellular metabolism and DNA presence (Walters *et al.* 2009). This is consistent with ratios between method results being farthest from unity at Avalon, where the fecal source is contaminated groundwater that is filtered through the sand before reaching the beach, thus increasing decoupling time and opportunity (Boehm *et al.* 2003). It is also consistent with our finding that correlations between QPCR and culture-based results were stronger and ratios closer to unity (when data were truncated) when the berms at Doheny and Surfrider were open and fecal contamination was concentrated.

Time of day affected the relationship between culture and qPCR results inconsistently across beaches. Correlations between methods were strongest in the morning at all beaches, consistent with sunlight serving as an inactivation agent for *Enterococcus* spp. (Fujioka *et al.* 1981, Noble *et al.* 2004, Walters *et al.* 2009). Because DNA is not as sensitive to sunlight as culturable cells and does not degrade as quickly after ultraviolet exposure (Walters *et al.* 2009), ratios between culturable cells and CCE were expected to fall over the course of the day. At Doheny, the ratio between culture and QPCR results decreased over the course of the day, as expected. However, the ratio at Avalon increased as the day progressed, suggesting that other factors, such as tide, had a larger effect than sunlight (Boehm *et al.* 2003). When the tide is high or rising, its pressure prevents groundwater from contacting the beach. Low or falling tides allow contaminated groundwater to mix with beachwater, giving a fresh pulse of contamination.

The skewness of the QPCR and culture-based measurements of *Enterococcus* spp. concentrations differed among beaches. At Avalon, *Enterococcus* spp. concentrations were generally high, but at Doheny and Surfrider, concentrations were often low. Because method variability is greater when concentrations are low (Whitman *et al.* 2010), we truncated data to ensure that patterns we observed were not driven by these low concentration samples with high variability. Truncation also eliminated any bias that may have been introduced by assigning Enterolert non-detects a higher concentration (10 MPN/100ml) than membrane filtration or QPCR non-detects (2 CFU or CCE/100ml), a difference necessitated by the greater dilution of samples for Enterolert analysis. When data were truncated, correlations between methods were unchanged but the ratios between culture and QPCR result generally decreased. Patterns in the method ratios over time of day and across beaches were similar, regardless of the exclusion of data at low concentrations. However, truncation of the dataset did yield important differences in patterns when comparing data across berm status. This is likely because water quality was good, yielding ratios near unity, on nearly 80% of closed-berm days. On these days fecal pollution was not simply diffuse, but often below detection and comparisons between method results were not meaningful.

Relationships between methods were sometimes complicated by differences in the QPCR assays. Two QPCR methods were included in this study to identify differences caused by QPCR chemistry. TaqMan assays have been used historically to determine relationships between QPCR results and epidemiological outcomes. Newer Scorpion-based

QPCR assays have not been used in epidemiology studies, but have the practical advantage of being slightly faster. In this study, the EntScorp assay tended to give slightly higher measurements than EntTaq even though both assays targeted the same gene. Results from the QPCR assays were always significantly correlated, but the correlation coefficient varied slightly among beaches (data not shown). Because measured QPCR efficiencies were similar (90 to 100%), differences between the assays were potentially due to quantification method. Results from the EntTaq assay were quantified using the ΔΔCt method, which allowed correction for extraction loss and presumptive identification of PCR inhibition using the SPC. In the EntScorp assay, the SPC was only used as a guide for identifying both extraction loss and inhibition.

Implications

Recent work pooling data from 36 sites across the United States has suggested that an empirical relationship can be developed between QPCR and culture-based measurements of FIB, though the authors allow that this relationship may be influenced by local environmental factors (Whitman *et al*. 2010). Results from this study demonstrate that the method relationships vary both spatially and temporally and indicate that a single empirical relationship between method results will not be universally appropriate. If QPCR methods for measuring *Enterococcus* are adopted, new standards will need to be developed or the relationship between the methods will need to be assessed at each beach. At the very least, an understanding of sources of fecal pollution at individual beaches will be required to elucidate meaningful relationships between culture and QPCR results.

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Supplemental Information

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