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Evaluation of rapid methods and novel indicators for assessing microbiological beach water quality

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ABSTRACT

A broad suite of new measurement methods and indicators based on molecular measurement technology have been developed to assess beach water quality, but they have generally been subjected to limited testing outside of the laboratory in which they were developed. Here we evaluated 29 assays targeting a variety of bacterial, viral, and chemical analytes by providing the method developers with twelve blind samples consisting of samples spiked with known concentration of sewage or gull guano and negative controls. Each method was evaluated with respect to its ability to detect the target organism, absence of signal in the negative controls and repeatability among replicates. Only six of the 30 methods detected their targets in at least 75% of the samples while consistently determining the absence of the target in the negative controls. Among quantitative methods, QPCR for *Bacteroides thetaiotamicron* and *Enterococcus* detected by Luminex reliably identified all but one sample containing human fecal material and produced no false positive results. Among non-quantitative methods, the *Enterococcus* esp gene, the Bacteroidales human specific marker and culture-based coliphage were the most reliable for identifying human fecal material. We also found that investigator-specific variations of methods targeting the same organism often produced different results.

1. Introduction

Growth-based measurements of fecal indicator bacteria (FIB) have been the basis for US EPA's recreational water quality criteria for over 40 years. FIB are routinely measured as surrogates for human pathogens because they are easy to measure and epidemiological studies of water contact illness have demonstrated a relationship between concentrations of these indicators and human health outcomes (Cabelli et al., 1979; Cabelli et al., 1982; Pruss 1998; Wade et al., 2003).

Despite their wide use, growth-based measurement methods of FIB are limited in their ability to protect swimmers from exposure to waterborne pathogens. One limitation is the time-lag between sample collection and result. Growth-based measurements require 18–96 h to obtain results, with contaminated beaches remaining open during the processing period and reopening long after levels of indicator bacteria have dropped below regulatory limits. Additionally, culture measurements in most traditional water quality laboratories are limited to indicator organisms that can be easily grown in an aerobic environment. Unfortunately, the aerobic growth requirement promotes use of indicators that potentially regrow in the ambient environment (Whitman et al., 2003; Desmarais et al., 2002; Solo-Gabriele et al., 2000; Jiang et al.,

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2007; Yamahara et al., 2007), which can confound the desired relationship between FIB concentrations and human fecal sources (Colford et al., 2007).

Taking advantage of advances in molecular measurement technology (Noble and Weisberg 2005), researchers have developed a broad suite of potential new measurement methods and indicators. Some have focused on measuring present FIB using methods that produce results in two hours or less. Others have focused on measuring pathogens or alternative indicators that are more closely associated with human fecal sources or on identifying more specific genetic sequences within FIB that are indicative of the fecal source.

Many of these advances have undergone performance evaluations, but generally within the research laboratories in which they were developed. More importantly, the evaluations have typically been limited to assessing target identification using a monocultural laboratory stock in a simple matrix, rather than with samples that contain potential interferences and alternative target materials. Ultimately, most of these methods will need to be incorporated into epidemiological studies to establish relationship between indicator density and health risk, even for new methods that measure existing indicators because of differences in measurement target. However, incorporating new methods into an epidemiological study is an expensive proposition and preliminary performance characteristics are needed to prioritize which methods are sufficiently advanced for inclusion. Here we present such a screening for new methods measuring a variety of analytes that were being considered for inclusion in epidemiological studies examining swimming-related illness at beaches in southern California.

2. Material and methods

Twelve researchers performed 30 different assays in the study (Table 1). Researchers processed samples and conducted data analysis using their own operating procedures. Several participants performed methods that targeted the same organism, but the analytical protocols employed differed substantially

Table 1 – Target organisms, detection methods and sample volumes employed by researchers.

Researcher	Target	Method	Sample volume	Reference
1	*E. coli*	IMS-ATP[a]	100 ml	Bushon et al., 2009
1	*Enterococcus*	IMS-ATP	100 ml	Bushon et al., 2009
2	*Enterococcus*	Luminex	500 ml	Baums et al., 2007
3	*Enterococcus esp* gene	PCR	200 ml	Scott et al., 2005
4	Human-specific *Bacteroidales*	PCR	100 ml	Bernhard and Field, 2000
5	Human-specific *Bacteroidales*	QPCR	500 ml	Bernhard and Field, 2000
6*	Human-specific *Bacteroidales*	QPCR	8 L	Kildare et al., 2007
5	*B. thetaiotamicron*	PCR	500 ml	Carson et al., 2005
7	*B. thetaiotamicron*	QPCR	100 ml	Converse et al., 2009
5	*M. smithii nifH* gene	PCR	500 ml	Ufnar et al., 2006
2	*M. smithii nifH* gene	QPCR	500 ml	Ufnar et al., 2006
2	*M. smithii nifH* gene	Luminex	500 ml	Baums et al., 2007; Ufnar et al., 2006
5	Swine-specific methanogen	PCR	500 ml	Ufnar, Ufnar, et al., 2007
5	Ruminant-specific methanogen	PCR	500 ml	Ufnar, Wang, et al., 2007
4	Gull-specific bacterium	PCR	100 ml	Field, Unpublished
8	*Legionella* spp.	PCR	500 ml	Jonas et al., 1995; Miyamoto et al., 1997
8	*Legionella pneumophila*	PCR	500 ml	Wilson et al., 2003
2	F- coliphage	EPA 1601	250 ml	USEPA, 2001
2	F+ coliphage	EPA 1601	250 ml	USEPA, 2001
9	F- coliphage	Two-step enrichment	2 L	USEPA, 2001
9	F+ coliphage	Two-step enrichment	2 L	USEPA, 2001
9	F+ DNA coliphage	CLAT[b]	2 L	Love and Sobsey, 2007
9	F+ RNA coliphage	CLAT	2 L	Love and Sobsey, 2007
9*	Human Adenovirus	PCR	20 L	Jothikumar et al., 2005a
2	Human Enterovirus	rt-QPCR	500 ml	Gregory et al., 2006
10	Hepatitis A virus	rt-QPCR	500 ml	Houde et al., 2007
2	Norovirus	rt-PCR	500 ml	Jothikumar et al., 2005b
9*	Norovirus	rt-PCR	20 L	Jothikumar et al., 2005b
11	Human Polyomavirus	PCR	600 ml	McQuaig et al., 2006
12	Optical Brighteners	Fluorometry	3 ml	Cao et al., 2009

*Did not process duplicates due to time constraints associated with large volume filtration.
a Immunomagnetic separation-ATP.
b Culture, latex agglutination, and typing (CLAT).

between researchers in terms of the volume and method filtering, method of DNA extraction, PCR primer set employed and method of target detection, and whether the result was qualitative (presence/absence) or quantitative. Hence, there were no true replicates between researchers and no attempt was made to standardize protocols or assess variability between researchers targeting the same organism. Although some methods employed by study participants have not yet been published, detailed methodologies for a majority of the methods may be found in the publications referenced in Table 1.

Each researcher analyzed 12 blind water samples in duplicate for each method, except for researchers 6 and 9. Due to logistical and time constraints imposed by the large volume filtrations required, Researcher 6 analyzed only a singlet 8 L samples for human-specific *Bacteroidales*. Similarly, Researcher 9 analyzed only singlet 20 L samples for human adenovirus and human norovirus. The same 12 samples were analyzed in duplicate by the Orange County Sanitation District laboratory using EPA Method 1600 *Enterococcus* and EPA Method 1603 for *Escherichia coli*.

The twelve samples consisted of three sample types (Table 2). Five were clean offshore seawater inoculated with different concentrations of human sewage. Sewage for inoculation was collected from the primary wastewater stream of the Orange County Sanitation District's Plant #2, which serves approximately six million people, and spiked into clean seawater collected 11 km offshore at a location presumed to be free of fecal contamination. These samples were intended to assess the sensitivity of methods to detect varying concentrations of their target analyte. They also served as negative controls for assays targeting gulls, ruminants, and swine.

Two samples were ambient water from Doheny State Beach inoculated with sewage. Freshwater was collected upstream in San Juan Creek and saltwater was collected in the ocean at the confluence of the creek and ocean. These samples were intended to determine if matrix constituents in the creek or beach water interfered with assays.

Three samples were negative controls that contained no fecal material. The first negative control was sterile phosphate-buffered saline. The second negative control was clean offshore seawater collected as described above. The third negative control was clean beach water collected at Imperial Beach, CA.

Two samples consisted of clean offshore seawater inoculated with gull guano. One was inoculated with guano collected from gulls at Doheny State Beach as described in Griffith et al. (2003) and was intended as a negative control for methods that target exclusively human sources. The other was inoculated with gull guano collected from long-term residents of a local wildlife rehabilitation facility. This sample was included because we could not be certain that the gulls at Doheny State Beach did not consume human fecal material from a nearby landfill (8 km away) or wastewater treatment facility (2 km) away that could conceivably cause their guano to be positive for a human marker. While cross-contamination with fecal material from their human caretakers cannot be ruled-out, gulls from the rehabilitation facility fed a prepared diet were considered much less likely to be cross-contaminated with a human fecal marker from their food source than those with free access to diapers and the like at landfills and human sewage in settling tanks at wastewater treatment facilities.

Samples were inoculated by placing water in sterile carboys and adding inoculants with stirring as described in Griffith et al. (2003). For sewage, influent was added in volumes intended to produce a range of indicator bacteria concentrations between 50 and 1×10^4 *Enterococcus* or *E. coli* per 100 ml. Four samples were created by inoculating gull guano (Wetland and Wildlife Care Center of Orange County, Huntington Beach, CA) into offshore seawater and Doheny Beach water. Approximately 1 g of gull guano was added to 10 L of seawater. Previous research conducted on similar fecal samples had shown that this inoculation should achieve a total Enterococcus sp. concentration of approximately 1000 cells per 100 mL (Griffith, unpublished data).

The study took place April 11–12, 2007 at the Orange County Sanitation District Environmental Laboratory in Fountain Valley, CA. Samples were created or collected between 6:00 and 9:00 AM each day and distributed to researchers to begin processing at 11:00. Each researcher, with the exception of Researchers 2, 4 and 9, performed filtrations on site and transported or shipped filters back to their laboratory for analysis. Water for coliphage analysis was shipped to Researchers 2 and 9 at their respective laboratories in Chapel Hill, NC and Charleston, SC. 20 L samples for virus analysis were also shipped to Researcher 9. SCCWRP personnel performed filtrations per instructions for Researcher 4 and shipped the filters to the researcher's lab for analysis. Due to a logistical issue, seawater samples inoculated with sewage

Table 2 – Average concentration of *E. coli* and *Enterococcus* in the blind samples.

	Sample	*E. coli* (cfu/100 ml)	*Enterococcus* (cfu/100 ml)
Negative Controls	PBS	<1	<1
	Imperial Beach shoreline	<2	<2
	Offshore seawater	<2	<2
Positive Controls	Offshore seawater spiked w/sewage (level 5)	6500	5500
	Offshore seawater spiked w/sewage (level 4)	860	1090
	Offshore seawater spiked w/sewage (level 3)	330	85
	Offshore seawater spiked w/sewage (level 2)	45	57
	Offshore seawater spiked w/sewage (level 1)	25	61
	Doheny Beach seawater spiked w/sewage	580	320
	San Juan Creek freshwater spiked w/sewage	>2000	>2000
Gull Samples	Doheny Beach seawater w/gull guano	>200,000	>200,000
	Offshore seawater w/captive gull guano	>2000	>2000

were not analyzed for the gull-specific genetic marker. Likewise, Researcher 9 did not analyze two samples for coliphage.

Methods were evaluated relative to four criteria. The first was specificity, which was defined as the ability of the methods to detect their target in the sewage or guano-spiked samples and correctly produce negative results for the control samples. Specificity was assessed by the percentage of sewage-spiked samples or negative controls correctly identified. The second criterion was sensitivity, which was defined as the ability of the methods to detect their target over a dilution series of sewage-spiked test samples. The third criterion was repeatability. As many of the methods were non-quantitative, repeatability was assessed as the percentage of duplicate samples that yielded the same result with respect to presence/absence of the target. Finally, for the methods that focused on source identification, the fourth criterion was whether they correctly differentiated samples that contained human fecal material from those that contained gull fecal material.

3. Results

Concentrations of *Enterococcus* and *E. coli* in positive controls ranged from non-detect to more than 200,000 cfu/100 ml for the sample spiked with guano (Table 2). Enterococcus concentrations in samples spiked with sewage ranged from 61 to 5500 cfu/100 ml. The three samples used as negative controls (sterile PBS, offshore seawater and beach water) all had non-detectable levels of FIB.

Among rapid methods targeting traditional FIB, the Luminex method for *Enterococcus* exhibited the highest specificity and sensitivity (Table 3). Luminex correctly detected *Enterococcus* in all but the most dilute sample and produced no false positive results for negative controls. In contrast, the IMS-ATP method for *E. coli* and *Enterococcus* was highly repeatable, but also exhibited a high rate of false positive results among negative controls for both indicator organisms.

Among putative human-specific indicator methods with bacterial targets, the *Enterococcus esp* genetic marker exhibited excellent specificity across all matrices and had no false positive results for negative controls (Table 3). *Bacteroides thetaiotamicron* by QPCR performed nearly as well, exhibiting similar repeatability (Table 4) and identifying all sewage-spiked samples, except for one duplicate of sewage spiked into San Juan Creek water, and produced no false positive results. The three methods for human-specific *Bacteroidales* produced very different results. The method carried out by Researcher 4 performed the best, correctly identifying 80 percent of sewage spiked samples, including all the matrix controls, with no false positives. The method performed by Researcher 5 had similar sensitivity for sewage-spiked samples in clean seawater and

Table 3 – Percentage of sewage-spiked samples and negative controls correctly identified.

Researcher	Target	Method	Sewage Spiked into Clean Seawater	Sewage Spiked into Ambient Water	Negative Controls
1	*E. coli*	IMS-ATP	100	100	33
1	*Enterococcus*	IMS-ATP	100	100	33
2	*Enterococcus*	Luminex	80	100	100
3	*Enterococcus esp* gene	PCR	100	100	100
4	Human-specific *Bacteroidales*	PCR	80	100	100
5	Human-specific *Bacteroidales*	QPCR	80	50	100
6	Human-specific *Bacteroidales*	QPCR	100	100	0
7	*B. thetaiotamicron*	PCR	100	25	50
7	*B. thetaiotamicron*	QPCR	100	75	100
5	*M.smithii nifH* gene	PCR	20	0	100
2	*M.smithii nifH* gene	QPCR	60	100	83
2	*M.smithii nifH* gene	Luminex	90	75	83
5	Swine-specific methanogen	PCR	0	0	100
5	Ruminant-specific methanogen	PCR	0	0	100
4	Gull-specific bacterium	PCR	not analyzed[a]	50	100
8	*Legionella spp.*	PCR	100	100	33
8	*Legionella pneumophila*	PCR	0	25	100
2	F- coliphage	EPA 1601	100	100	100
2	F+ coliphage	EPA 1601	75	100	100
9	F- coliphage	two-step enrichment	100	100	67
9	F+ coliphage	two-step enrichment	30	100	100
9	F+ DNA coliphage	CLAT	20	50	100
9	F+ RNA coliphage	CLAT	30	50	100
9	Human Adenovirus	PCR	40	50	67
2	Human Enterovirus	rt-QPCR	20	25	100
10	Hepatitis A virus	rt-QPCR	0	0	100
2	Norovirus	rt-PCR	80	0	100
9	Norovirus	rt-PCR	0	0	100
11	Human Polyomavirus	PCR	80	50	100
12	Optical Brighteners	fluorometry	0	0	100

a Sewage – spiked samples not analyzed for gull marker due to logistical issues.

Table 4 – Percentage of samples with consistent results between duplicates.

Researcher	Target	Method	Consistent Binary Results
1	*E. coli*	IMS-ATP	100
1	*Enterococcus*	IMS-ATP	100
2	*Enterococcus*	Luminex	89
3	*Enterococcus esp* gene	PCR	89
4	Human-specific *Bacteroidales*	PCR	89
5	Human-specific *Bacteroidales*	QPCR	89
5	*B. thetaiotamicron*	PCR	100
7	*B. thetaiotamicron*	QPCR	85
5	*M.smithii nifH* gene	PCR	100
2	*M.smithii nifH* gene	QPCR	67
2	*M.smithii nifH* gene	Luminex	61
5	Swine-specific methanogen	PCR	100
5	Ruminant-specific methanogen	PCR	100
3	Gull-specific bacterium	PCR	100
8	*Legionella* spp.	PCR	100
8	*Legionella pneumophila*	PCR	83
2	F- coliphage	EPA 1601	100
2	F+ coliphage	EPA 1601	81
9	F- coliphage	Two-step enrichment	100
9	F+ coliphage	Two-step enrichment	61
9	F+ DNA coliphage	CLAT	61
9	F+ RNA coliphage	CLAT	61
2	Human Enterovirus	rt-QPCR	94
10	Hepatitis A virus	rt-QPCR	94
2	Norovirus	rt-PCR	89
11	Human Polyomavirus	PCR	94
12	Optical Brighteners	fluorometry	100

excellent repeatability, but did not detect one of the matrix controls and exhibited a 50 percent false positive rate for negative controls. The human-specific *Bacteroidales* assay performed by Researcher 6 correctly identified all the sewage-spiked samples, but failed to differentiate between spiked-samples and negative controls.

Of three assays that targeted *Methanobrevibacter smithii*, the Luminex version correctly identified all but one of the sewage-spiked samples, but also produced one false positive result. Both the QPCR and PCR methods for *M. smithii* were much less sensitive than was the Luminex method, identifying only 60 and 20 percent of sewage spiked samples, respectively. Repeatability of the Luminex and QPCR assays for *M. smithii* was poor (Table 4).

Neither of the assays targeting *Legionella* spp. performed well as indicators of sewage: While the genus-based assay was able to identify all of the sewage-spiked samples, it exhibited a high rate of false positive results for the negative controls. In contrast, the species-specific assay for *Legionella pneumophila* produced no positive results.

Among the animal specific bacterial assays, only the gull marker produced positive results. This method correctly identified all samples spiked with gull guano. It also returned a positive result for the sewage-spiked matrix control sample collected from San Juan Creek, but this might reflect the large number of gulls observed in the creek at the time the water was collected. Gull, ruminant and swine marker assays were otherwise negative for all other samples (data not shown).

Both somatic (F−) coliphage methods produced similar results in terms of sensitivity, but differed in that EPA Method 1601 had superior specificity and repeatability than the two-step enrichment method. For male-specific (F+) coliphage, Method 1601 also exhibited far superior sensitivity than the two-step enrichment method. Specificity of both methods was excellent and no matrix effects were observed. When the ten samples positive for F+ coliphage by the two-step enrichment method were assayed using the CLAT method, 9 tested positive for Type I F+ RNA coliphage and 3 out of these 9 were also positive for F+ DNA coliphage.

Among methods that targeted human viruses, both the polyomavirus assay and the norovirus assay performed by Researcher 2 detected 80 percent of sewage-spiked samples. The norovirus assay was slightly more sensitive, detecting the lowest level of sewage in the clean seawater matrix, but was unable to detect sewage when spiked into ambient waters. In contrast, the human polyoma virus method was able to detect half of the ambient water samples spiked with sewage, but was less repeatable than was the norovirus assay. The human adenovirus and enterovirus assays were much less sensitive, detecting only the higher concentrations of sewage in spiked samples and returning no positive results for the sewage-spiked ambient samples. Two methods, the norovirus assay performed by Researcher 9 and the Hepatitis A virus assay, did not produce positive results for any of the sewage-spiked samples.

The optical brightener assay fared poorly in this study. While it correctly identified all of the negative controls, it was unable to detect even the highest concentration of sewage in the spiked samples.

4. Discussion

Only six of the 30 methods detected their targets in at least 75% of the samples while also consistently determining the absence of the target in the negative controls. Of the methods that target human-specific fecal material, the *Bacteroidales* human specific marker performed by Researcher 4 fared among the best, which is consistent with previous studies. For example, in an evaluative study comparing microbial source tracking methods Griffith et al. (2003), this method outperformed all others in identifying samples containing human fecal material. Subsequent studies in Australia (Ahmed et al., 2008a) and Europe (Gourmelon et al., 2007) have confirmed the utility of this method for indentifying human sources of fecal contamination. We also found that *B. thetaiotamicron* performed well. This is the first independent demonstration of the specificity of this marker for human fecal material using blind samples. In a previous study, this marker demonstrated excellent sensitivity with human sources, although some cross-reactivity was observed with dogs Carson et al. (2005).

The *esp* gene has received mixed reviews in previous studies, but fared well in our study. Layton et al. (2009) found the *esp* gene to be widespread in a variety of mammals and birds. In contrast, Whitman et al. (2007) identified the gene in less than 10% of the non-human animals they tested, but in 90% of sewage samples. In a separate study conducted in Australia, Ahmed et al. (2008b) also observed the marker in greater than 90% of sewage samples, but did not find it in any of the animals they tested, leading them to conclude that it was sewage-specific. One reason the *esp* gene may have performed better in our study than in Layton et al. is that we used sewage as the main inoculum for our test samples. The only opportunity to observe a false positive result was in the samples spiked with gull guano, making this a less than optimal test for cross-reactivity with other sources.

We also found that coliphage performed well, which is consistent with its epidemiological performance (Colford et al., 2007) and in studies of treated wastewater and surface waters impacted by human sewage (Dhillon et al., 1970; Paul et al., 1997; Havelaar et al., 1986). Although somatic (F-) coliphage performed as well as male-specific (F+) coliphage, F+ coliphage may have greater utility as an indicator because it is amenable to additional typing analyses that allow differentiation between human and animal sources of contamination (Cole et al., 2003; Love and Sobsey 2007).

Enterococcus measured by Luminex identified all but one sample containing fecal material and produced no false positive results. There have been a number of publications documenting the success in rapid enumeration of *Enterococcus* using QPCR quantified by fluorescent probes (Haugland et al., 2005; Noble et al., 2009; Wade et al., 2008), but only one using Luminex (Baums et al., 2007). However, the Luminex system offers a potential advantage in that in is capable of simultaneously enumerating multiple indicators in a single assay. Despite its promise and wide use in medical research, the system has yet to be fully exploited for water quality monitoring.

Of the methods that did not fare well, five incorrectly identified the presence of their target in 50% or more of the negative control samples. Two of these were antibody-based methods for measuring *E. coli* and *Enterococcus*. Antibody methods are dependent on broad cellular recognition patterns which can be less species-specific than genetic targets. There are many naturally occurring marine bacteria, including gram positive cocci and many others which have yet to be characterized, that might have sufficiently similar surface properties to cause a false positive. That these methods correctly identified the absence of target in the PBS controls, but erred in the two seawater controls, is consistent with possible non-specific binding of antibodies with native marine bacteria.

Although the optical brightener method did not detect human sewage in any of the test samples, this finding is inconsistent with a previous study which used sewage from the same source (Cao et al., 2009). This method, though, has been more focused on detecting septage in stream water where its fluorescent target is more concentrated than in the sewage-spiked samples used in this study.

Some methods that performed well with sewage spiked into offshore seawater had difficulties when sewage was spiked into nearshore water. This probably reflects the sensitivity of PCR-based methods to interference from inhibitory matrix constituents, such as humic acids and complex carbohydrates that are more likely to occur in nearshore waters. Interestingly, this was of lesser concern for methods that included a growth step. For instance, somatic coliphage measured by EPA Method 1601 correctly classified all samples. Similarly, the *esp Enterococcus* marker correctly classified all samples, even though it is based on PCR. However, it has an initial step in which EPA Method 1600 is used to select and grow enterococci that are subsequently washed from the membrane and collected prior to amplification (Scott et al., 2005). It is possible that the growth and washing steps act to dilute or leave PCR inhibitory compounds behind on the membrane.

While not all methods performed well, the results need to be interpreted in context of our study design. For example, many of the source-specific markers were not developed in California and it is possible that geographic differences in microbial populations may have contributed to reduced sensitivity or false positive results caused by organisms not present in the locale where the method was developed. In addition, the use of human sewage and gull guano as inoculants was adequate for most, but not for all methods tested. For instance, municipal sewage from large human populations routinely tests positive for human viruses, but not necessarily at densities that are quantifiable in a small volume sample, particularly for rare viruses such as Hepatitis A. It is possible that the virus methods correctly capture their targets and would have identified their presence in more samples if we had inoculated with a higher concentration of sewage or with a suite of live human pathogens, but the sewage concentrations we used and the volumes we provided for measurement are typical of those used for routine beach water quality monitoring.

We also found that different assays targeting the same organism can produce very different results. For example, of two methods targeting *B. thetaiotamicron*, the QPCR method far outperformed the non-quantitative method in both specificity and robustness. Among-researcher variability was similarly high among the three *M. smithii* methods, where increased sensitivity and robustness of the quantitative methods was offset by reduced specificity. Some of this difference is due to variations in the method themselves, but some of the difference may also have to do with implementation. For instance, two of the method variants targeting human-specific *Bacteroidales* produced similar results, while the third method produced positive results for all test samples, including the negative controls. Subsequent investigation by this method developer led to the discovery of a previously undetected problem of target DNA carry-over across samples in the re-usable hollow-fiber filter apparatus used to concentrate samples prior to QPCR quantitation. While unfortunate, this discovery provided the impetus for improvements to the filtration method and more rigorous cleaning procedures have since been instituted (S. Wuertz, personal communication). Thus, poor performance by an individual method variant or analyst in our testing does not mean that the method could not be made to work under other circumstances.

5. Conclusions

- Only the *Enterococcus* measured by Luminex; *Enterococcus esp* gene; human-specific Bacteroidales (Researcher 4); B. thetaiotamicron (Researcher 7); F- coliphage (Researcher 2); and F+ coliphage (Researcher 2) methods detected their targets in at least 75% of the samples while consistently determining the absence of the target in the negative controls.
- Among quantitative methods, QPCR for *Bacteroides thetaiotamicron* and *Enterococcus* detected by Luminex reliably identified all but one sample containing human fecal material and produced no false positive results.
- The *Enterococcus* esp gene, the *Bacteroidales* human specific marker and culture-based coliphage were the most reliable among non-quantitative methods.
- Investigator-specific variations of methods targeting the same organism often produced different results.

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Evaluation of rapid methods and novel indicators for assessing microbiological beach water quality

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Abstract

A broad suite of new measurement methods and indicators based on molecular measurement technology have been developed to assess beach water quality, but they have generally been subjected to limited testing outside of the laboratory in which they were developed. Here we evaluated 29 assays targeting a variety of bacterial, viral, and chemical analytes by providing the method developers with twelve blind samples consisting of samples spiked with known concentration of sewage or gull guano and negative controls. Each method was evaluated with respect to its ability to detect the target organism, absence of signal in the negative controls and repeatability among replicates. Only 6 of the 30 methods detected their targets in at least 75% of the samples while consistently determining the absence of the target in the negative controls. Among quantitative methods, QPCR for *Bacteroides thetaiotamicron* and *Enterococcus* sp. detected by Luminex reliably identified all but one sample containing human fecal material and produced no false positive results. Among non-quantitative methods, the *Enterococcus* esp gene, the Bacteroidales human specific marker and culture-based coliphage were the most reliable for identifying human fecal material. We also found that investigator-specific variations of methods targeting the same organism often produced different results.

Due to distribution restrictions, the full-text version of this article is available by request only.

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