Evaluation of new, rapid microbiological methods for measuring recreational water quality

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ABSTRACT - Public health officials routinely measure fecal indicator bacteria to assess beach water quality, but present laboratory methods require about 24 hours to obtain a result, delaying issuance of health warnings until the day following sampling. New, more rapid measurement methods that would allow for same day warnings are under development, but have not yet undergone independent testing. Here we evaluate four of these new methods: immunomagnetic separation coupled with ATP bioluminescence, flow cytometry, quantitative polymerase chain reaction (Q-PCR) and dual-wavelength fluorimetry (DWF). Testing was conducted in two phases. In the first phase, developers of each new method processed 54 blind samples. Results were then compared to those produced by five laboratories using two traditional methods of analysis. Samples included both natural and laboratory-created samples, ensuring method evaluation over a range of concentrations, matrices and interferences. None of the new methods performed well enough to replace existing methods at present, but there were encouraging results for two methods. Q-PCR produced results within the range of the reference labs for two-thirds of the samples, but overestimated others, particularly those with complex matrices. DWF had the best precision among the new methods and was more precise than some of the laboratories using traditional methods, but also produced results that were generally higher than existing methods, especially for samples that contained urban runoff. In the second phase of testing, practitioners of traditional culture-based methods were trained in the new methods and asked to process nine blind samples. These practitioners produced results comparable to those produced by the method developers. While there is need for performance improvement in each of the new methods, technology transfer does not seem to be an important impediment to their adoption.

INTRODUCTION

California's public health officials routinely measure fecal indicator bacteria to assess recreational water quality. State Health Department regulations require weekly measurements of indicator bacteria (total coliforms, fecal coliforms, and enterococci) at high-use beaches. These regulations further require that the public be warned of possible health risks if any of these bacterial indicators exceed threshold values that were established through epidemiological studies.

California's investment in these water quality-monitoring programs could be improved if the laboratory time for enumerating bacteria was shortened. United States Environmental Protection Agency approved methods for measuring indicator bacteria in recreational waters require an 18- to 96-h incubation period, while several recent studies have shown that temporal changes in indicator bacteria levels in beach water occur much more rapidly (Leecaster and Weisberg 2001, Boehm *et al.* 2002). Thus, contaminated beaches can be open during the incubation period and be clean by the time warnings are posted. This time lag also inhibits tracking of contamination sources, since the signal can dissipate before upstream tracking is initiated. Lacking a more rapid method, investigators are unable to follow the trail of contamination back to its origin.

Technological advances provide new opportunities for measuring bacteria more rapidly. Whereas present methods rely on culturing bacteria and measuring growth or metabolic activity, new methods allow direct measurement of cellular attributes such as genetic material or surface immunological properties. Removing the extended incubation step allows these methods potentially to provide results in less

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than 4 h, with sufficient time for managers to take action to protect the public health (i.e., post warning signs or close a beach) on the same day that water samples are collected.

Genetic and immunological methods have advanced considerably for use in disciplines such as the food service and hospital industries (Fung 2002, NRC 2004). Effort has recently begun to focus on application of these new methods for recreational water quality testing (Noble and Weisberg in press). Water testing presents challenges not frequently encountered in other fields, such as complex sample matrices and the presence of other potentially confounding native bacterial species. As such, extensive testing of these methods is needed to ensure that they provide comparable reliability to the culture-based methods they are intended to replace. Here, we present a study evaluating four new rapid methods to assess whether they are suitable replacements for existing methods.

METHODS

Four new rapid methods were tested. The first was immunomagnetic separation coupled with ATP Bioluminescence (IMS/ATP). The IMS/ATP method uses magnetic beads coated with antibodies specific to *Enterococcus*. to bind and capture the target bacteria in a water sample. Once bound, the magnetic bead/antibody/bacteria complexes are pulled out of solution using a powerful magnet. Now separated from the rest of the bacterial population, the captured cells are enzymatically-lysed, releasing their ATP into solution. This ATP is then quantified via a bioluminescent assay. The amount of ATP is converted to the number of bacteria captured following a calibration curve established using stock cultures of known concentration.

The second was a flow cytometry (FC) method, which employed the Advanced Analytical Technologies (Ames, IA) RBD 3000 instrument. Like IMS/ATP, this method derives its specificity from antibodies specific to *Enterococcus*. Once captured by the first antibody, the target cells are labeled using a secondary antibody containing a fluorogenic tag that, when excited by a laser in the instrument, allows the cells to be enumerated in the flow cytometer.

The third method was quantitative polymerase chain reaction (Q-PCR), which detects and enumerates unique genetic targets found in *Enterococcus*. The bacteria are first captured on a filter. The filter containing the bacteria is then subjected to bead beating, which mechanically lyses the cells and releases their DNA into solution. This DNA then is used in the quantification step, where enterococcal DNA is simultaneously amplified and measured using the Taqman® system of fluorescent probes (Applied Biosystems, Foster City, CA) and the advanced optics of the Q-PCR instrument (Cepheid, Sunnyvale, CA).

The last method was dual-wavelength fluorimetry (DWF), which relies on the same sugar-fluorophore substrate as is used in the commercially available IDEXX™ assays, but advances the detection process. Through use of a novel fluorometer, the method simultaneously measures the rate at which bacteria take up the chromogenic substrate as well as the rate at which the fluorescent byproduct of substrate metabolism appears. This ratiometric measurement allows detection and enumeration of target bacteria in a matter of only four hours.

Evaluation Approach

The evaluation approach was to assess equivalency with existing methods through simultaneous processing of water samples using both new and existing methods of enumerating fecal indicator bacteria. Samples processed included both natural samples and laboratory-created samples, to ensure that a range of conditions was evaluated. Laboratory-created samples were included because they offer the ability to control the number of indicator organisms and potentially interfering contaminants present, but they do not completely mimic natural conditions. Environmental water samples were included because they contain complex combinations of interferences that cannot be duplicated in artificial samples, though they offer less control over specific variables that need to be evaluated.

Testing occurred in two phases. The first phase involved application of the new methods by the experts that developed them. The second phase involved application of the methods by senior members of several local microbiology laboratories, who would become likely users of these methods should they be approved. The goal of the second phase was to assess whether these new technologies are readily transferable to local practitioners.

In phase one, participants analyzed 54 blind samples consisting of triplicates of each of 18 different test samples. Sample processing occurred over 3 d, with triplicates of each of six samples processed

on each day. Processing occurred over 3 d because participants identified that 18 samples were the most they could analyze within the 4-h time frame without duplicative equipment and personnel.

Six of the eighteen Phase I samples consisted of laboratory-created samples in a seawater matrix inoculated with differing levels of fecal contamination. Seawater used to prepare these samples was collected from 5 km offshore in an area known to be free from allochthonous fecal contamination. Three of these samples were inoculated with treated wastewater effluent from the Orange County Sanitation District (OCSD) in Fountain Valley, California. Three were inoculated with urban runoff collected from the Seventh Street drain, flowing into the Los Angeles River.

Four additional samples were a seawater matrix inoculated with sewage effluent, but with a more complex matrix. Two of these were created using the offshore seawater and OCSD effluent, but mixed humic acids (Sigma, St. Louis, MO) added at a concentration of 0.001% w/v. The other two were prepared using seawater collected at a nearshore location known to have historically high levels of suspended solids, but low levels of fecal bacteria. The inoculum for these samples was effluent from the Los Angeles County Sanitation District's Carson wastewater treatment plant.

Four samples were natural samples, including a shoreline sample collected at Doheny State Beach in Dana Point, California, and urban runoff samples from three freshwater locations: Yorktown Drain in Huntington Beach, California; Santa Ana River in Fountain Valley, California; and San Juan Creek in Dana Point, California.

The last four samples were different types of blanks. These consisted of sterile phosphate-buffered saline (PBS); uninoculated offshore seawater, 0.2 μm filtered offshore seawater, and 0.2 μm filtered urban runoff from Yorktown Drain.

Nine samples were processed in Phase II, in which local practitioners implemented the new methods. These consisted of triplicates of each of three different test samples in a seawater matrix. These samples were prepared as in Phase I, inoculating OCSD effluent into offshore seawater. All samples were blinded.

Phase I testing took place on June 2, 3, and 4, 2004. Samples were created or collected between 6:00 a.m. and 9:00 a.m. each day and distributed by 11:00 a.m. to each of the five local labs that used routine methods. All participants began processing samples at the same time and processed samples in numbered order to minimize any concentration differences that might have developed from degradation during sample transport or laboratory holding.

Phase II testing was conducted on June 9, 2004. Local users, each a senior member of a large southern California microbiology lab, were each assigned to conduct one of the methods. Developers of new methods provided the local users with a written Standard Operating Procedure (SOP) and basic training in the operation of the equipment prior to the exercise. These users also learned by observing, and assisting as appropriate, during the Phase I testing.

During both phases, all samples also were processed by five local laboratories (Table 1) using methods they employ in their routine processing. For enterococci, this included both Enterolert® chromogenic substrate (CS) (IDEXX, Westbrook, ME) and membrane filtration (MF) methods.

Data analysis

The primary means of data analysis was to compare the results from the new methods to that from traditional methods employed by the reference laboratories. This was done in three ways. First, we assessed the number of individual samples from each new method that differed by half a log unit from the reference laboratory median. Half a log unit was selected because previous laboratory intercalibration

Table 1. Local laboratories that analyzed the test samples using presently approved methods.

Laboratory	MF Method	CS Method
Los Angeles County Sanitation Districts	EPA 1600	Enterolert Quantitray® 2000
City of Los Angeles	Std. Methods 9230 C.	Enterolert Quantitray® 2000
Orange County Sanitation District	Did not perform MF	Enterolert Quantitray® 2000
Orange County Public Health Laboratory	EPA 1600	Enterolert Quantitray® 2000
City of San Diego	EPA 1600	Enterolert Quantitray® 2000

studies (Noble *et al.* 2004; Griffith *et al.* 2004) have demonstrated that this is the typical range of variability observed for traditional methodologies, both within and among laboratories. For this analysis, blank samples were counted as outside of range when values exceeded 50 cells/100 ml.

Second, results were individually evaluated for false positives and false negatives relative to the State of California standard of 104 cells/100 ml, as the State requires posting warning signs for any sample that occurs above this level. The decision of whether a sign should have been posted that day, against which the new methods were being evaluated, was based on the median concentration for that sample as measured by the reference laboratories.

The final analysis assessed precision of the measurements, which we could do because each sample was processed three times as blind replicates. Precision was quantified as the average coefficient of variation (CV) and was compared between the new methods and the reference laboratories. In all cases, the CV was calculated using the standard deviation and mean across all sample types for each method or reference laboratory. For purposes of data analysis, qualifiers (< or >) were ignored and only the numerical value was used.

RESULTS

Q-PCR

Of the methods evaluated, Q-PCR was the most comparable to the reference labs, exhibiting the lowest rate of false positives (Figure 1) and the fewest number of samples more than 1/2 log unit from the reference laboratory median (Table 2). This method performed best on samples that contained moderate to high levels of enterococci in a simple seawater matrix without added interferences. However, Q-PCR tended to overestimate levels of enterococci, producing a higher mean value than traditional methods for two-thirds of the samples (Table 3).

Interferences from humic acids were not evident with Q-PCR, perhaps because the polycarbonate filters used do not retain them. Interferences from suspended solids were observed. With exception of Yorktown drain, results from the natural water samples showed good agreement between Q-PCR and reference method analyses (Tables 2 and 3).

Q-PCR exhibited relatively poor precision between replicate samples (Table 4), particularly when levels of enterococci were low. The greatest variability was observed for blanks (including filtered samples) and seawater samples containing interferences. Variability was lowest for samples containing a simple seawater matrix spiked with sewage.

Comparability to existing methods was about the same when Q-PCR was performed by local users in Phase II, as it was in Phase I. Q-PCR overestimated slightly relative to existing methods, but not by enough to cause false positives and within the measurement error typically encountered within existing methods (Table 5). Precision during Phase II was even better than when conducted by the Phase I method experts (Table 4).

Local practitioners who performed the Q-PCR method during Phase II found it to be relatively simple to complete. Sample analysis from start to finish took about 3 h for the 9 samples. Despite the speed with which the analysis was completed, several hours of preparatory work were required to perform tasks such as labeling tubes, preparing reagents, and setting up equipment. Participants felt that the amount and difficulty level of the preparatory tasks were similar to that required to perform standard membrane filtration analyses.

Dual Wavelength Fluorimetry

Comparability of DFW to the reference methods was sample dependent. The method exhibited a high

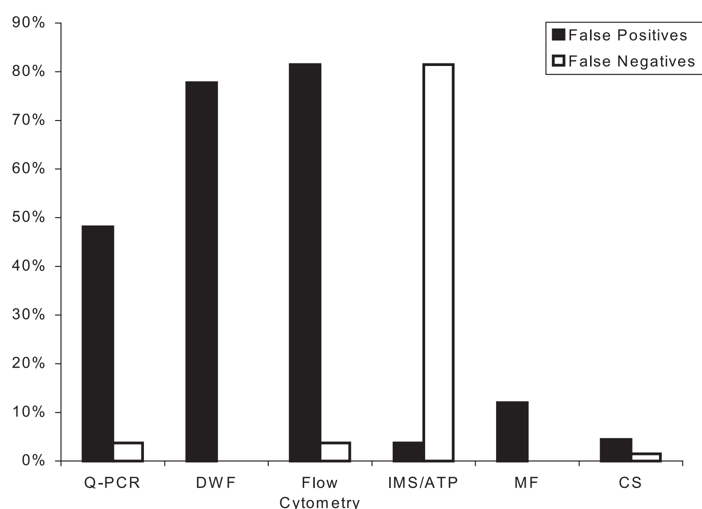

Figure 1. Percentage of false positives and false negatives with respect to California's warning threshold of 104 cfu/ 100 mL based on comparison to the reference laboratory grand median.

Table 2. Percentage of samples differing by more than fi log unit from the grand median produced by reference laboratories during Phase I.

Method	Seawater with Sewage n=9	Seawater with Urban Runoff n=9	Seawater with Interferences n=12	Natural Samples n=12	Total n=42
IMS/ATP	100	89	92	83	90
Flow Cytometry	67	100	25	42	62
DWF	11	100	25	50	45
Q-PCR	0	33	42	25	33

number of false positives (Figure 1), particularly for samples containing urban runoff either as an inoculum into seawater or as a natural sample. It was most comparable on samples consisting of a natural seawater matrix with moderate to high levels of bacterial contamination (Tables 2 and 3).

DFW displayed the best precision among the new methods tested. Variability of results for replicate samples during both Phase I and Phase II was comparable to that of the reference laboratories (Table 4).

Potential end-users that performed DFW during Phase II liked it because of its similarity to the chromogenic substrate methods they currently employ in their laboratories. However, they found that performing the method was labor intensive and could be somewhat tedious. The main reason was that the operator is required to transfer each sample back and forth between the water bath incubator and the measurement device multiple times per hour during the analyses, and so must remain close to the instrument. Users also observed that in its current configuration, the instrument required considerable set-up time prior to starting sample analysis. Despite this, the novice operators had a positive outlook on the future of the DWF method. They found the method to be straightforward to perform and felt that its operational shortcomings could be easily overcome by automating certain steps of the analysis protocol.

Flow Cytometry

The flow cytometry method consistently overestimated *Enterococcus* levels relative to present methods (Table 3), leading to a false positive rate relative to state standards of more than 50% (Figure 1). The problem appeared to be associated with high background counts, as values higher than state standards were measured in 11 of the 12 blank samples. This overestimation problem was also encountered in Phase II of the testing (Table 5).

Variability among replicate samples during Phase I was typically low and the method displayed superior precision except when presented with sterile phosphate buffered saline or urban runoff (data not shown). Variability during Phase II was comparable to that of the reference laboratories.

Potential end-users who performed this method during Phase II found it to be labor intensive. Of particular concern was the amount of time it took to complete all of the necessary steps in the protocol before the sample could be loaded into the instrument. As with DFW, there was a consensus among users that the method could be improved by automating many of the more labor-intensive steps.

IMS/ATP

IMS/ATP consistently underestimated levels of *Enterococcus* in the test samples (Tables 2 and 3), which resulted in a high rate of false negatives (Figure 1). This held true regardless of the different sample matrices, the presence of interferences, or whether the sample was natural or laboratory created. Variability among replicates in Phase I was high, but was similar to the reference laboratories during Phase II (Table 4).

Potential end-users liked the simplicity of the concepts behind this method and the fact that, unlike the other methods tested, it was designed for field use. However, they also found the method to be labor intensive and felt that some streamlining of the protocol would be necessary to adapt the method for processing multiple samples in the laboratory.

Discussion

Q-PCR was the most accurate of the methods, but it generally overestimated *Enterococcus* concentrations relative to the culture-based methods. This is consistent with previous Q-PCR applications in environmental water samples, where culture-based

Table 3. Mean Enterococcus values (cells/100 mL) produced by each method in Phase I.

Sample Description	IMS/ATP	Flow Cytometry	DWF	Q-PCR	Reference (MF)	Reference (CS)
Sterile Phosphate Buffered Saline	2	230	1	6	4	<10
0.2um filtered Offshore Seawater	1	179	6	40	4	<10
0.2um filtered Yorktown Drain	2	16	5,289	70	4	<10
Offshore Seawater	1	552	359	64	4	<10
Offshore Seawater w/Sewage (1)	1	1,068	269	93	228	91
Offshore Seawater w/Sewage (2)	1	1,575	194	255	490	239
Offshore Seawater w/Sewage (3)	1	6,648	1,471	1,486	5,942	3,147
Doheny Beach	481	4,048	>10,000	5,634	4,825	4,615
Offshore Seawater w/Sewage and Humic Acids (1)	6	1,828	784	1,023	1,082	697
Offshore Seawater w/Sewage and Humic Acids (2)	3	13,363	5,016	5,629	11,167	9,916
Seawater w/Sewage and Suspended Solids (1)	16	531	1,092	1,665	122	73
Seawater w/Sewage and Suspended Solids (2)	27	1,291	1,761	3,854	1,133	737
Offshore Seawater w/Urban Runoff (1)	8	703	5,929	80	39	34
Offshore Seawater w/Urban Runoff (2)	1	1,119	>10,000	578	86	80
Offshore Seawater w/Urban Runoff (3)	1	1,165	>10,000	539	79	72
Yorktown Drain	40	1,347	7,517	133	8	18
San Juan Creek	226	5,523	>10,000	7,477	8,267	6,074
Santa Ana River at OCSD Plant	13	244	8963	675	476	356

CS - Chromogenic substrate
MF - Membrane filtration

methods have rightly or wrongly been used as a kind of "gold standard", and may reflect the fact that measurements of non growth-related attributes of target bacteria do not differentiate between cultivable and non-cultivable cells (Duprey *et al*. 1997, Frahm and Obst 2003, Brinkman *et al*. 2003). This is also consistent with the greater success of this method with sewage-inoculated samples than with urban runoff inoculated samples, since sewage is a fresher fecal source and should have a higher percentage of cultivable cells.

While measurement of noncultivable cells is a likely factor in this method's overestimation, other possible explanations include specificity of the primer set to *Enterococcus* species. The primers used in this test have undergone only limited testing to assess possible overestimation due to detection of non-target organisms. Frahm and Obst (2003) found that the Ludwig and Schleifer (1994) assay, on which these primers are based, can detect other related genera, but with much lower sensitivity. Further testing with marine samples will be necessary to assess possible overestimation of enterococci due to detection of non-target species found in natural marine waters.

Another possible reason for overestimation by Q-PCR may be that it is a more inclusive measure of *Enterococcus* spp. than the culture based methods commonly employed in water quality laboratories, which measure enterococci based on phenotypic characteristics that allow only a subset of cells to proliferate under a particular set of biochemical and environmental conditions. (Alves *et al.* 2004, Harwood *et al.* 2004, Velasco *et al*. 2004, Domig *et al.* 2003). Newer PCR approaches are beginning to focus on primers for *Enterococcus* species such as *E. faecilis* or *E. faecium*, which are easier to define genetically and may be more specific to fecal sources than the larger *Enterococcus* group (Harwood *et al.* 2004, Santo Domingo *et al.* 2003).

There is also the possibility that the Q-PCR method is fundamentally sound but suffers from implementation issues. Cross-sample contamination is particularly problematic with PCR because many

Table 4. Average coefficient of variation by method across all samples for Phases I and II.

Method/Laboratory	Phase I	Phase II
IMS/ATP	0.60	0.14
Flow Cytometry	0.42	0.25
DWF	0.18	0.28
Q-PCR	0.57	0.35
Reference (CS)	0.17	0.20
Reference (MF)	0.23	0.27

CS - Chromogenic substrate
MF - Membrane filtration

copies of the DNA target are produced during each analysis and are typically unaffected by laboratory practices oriented toward minimizing transfer of live organisms (Wilson 1997). In this particular application, we observed several practices that could have led to cross-sample contamination, including use of 95% ethanol to clean forceps between handling sample filters (a deviation from the processing protocol, which called for flame sterilization), and the use of de-ionized water rinses followed by autoclaving to clean and sterilize filter funnels between samples. Ethanol is used widely in many DNA extraction and purification protocols and could have served to preserve DNA contamination. Autoclaving, while fatal to living organisms, does not destroy their DNA, which can then be carried over to the next sample (Simmon *et al.* 2004, Sambrook *et al.* 2001). Cross-sample contamination would explain the inconsistent response among blanks for this method and would also explain the high variability among replicates. However, some of the variability could also be attributable to small differences in DNA extraction efficiency between individual samples (Haugland *et al*. 1999, Haugland *et al.* 2002).

Further work will be necessary to assess the causes for overestimation, but overestimation by rapid methods is preferable to underestimation. Initially, rapid methods are most likely to be used in a screening manner to look for system upsets, such as a leaking sewer line, and a reliable method cannot miss such events. Overestimation is less critical because management decisions would rarely be made at the screening level unless indicator concentrations were extreme (NRC 2004). Even extreme bacterial concentrations would likely lead to visual examination of the system for sewage system leaks rather than immediate beach closure. Moderate concentrations would most likely lead to verification of the spatial and temporal extent of the problem, which could be initiated that same day using traditional methods. While there are costs associated with false positives, these costs may be outweighed by the extra protection against the extreme circumstance occurring at a high-use beach. Still, reduction of the false positive rate from its present level will probably be necessary before these methods are accepted for general use.

DWF had the best precision among the methods and in some cases, better than some of the laboratories using standard methods. This is particularly impressive, given that the precision of the reference laboratories in this study likely is better than that for most microbiology laboratories because each participated in at least three prior intercalibration studies designed to facilitate comparability among southern California laboratories (Noble *et al.* 2003, Noble *et al.* 2004, Griffith *et al.* 2004). Variability within and across laboratories prior to these exercises was typically higher than that observed for any of the new methods evaluated in this study.

While DWF was highly repeatable, it severely overestimated enterococcus counts in samples containing urban runoff, either as the matrix or as an inoculum. This was true even when the urban runoff had been passed through a 0.2 μm filter, suggesting that non-biological processes may be responsible for

Table 5. Mean Enterococcus values (cells/100 mL) produced by each method in Phase II.

Sample	IMS	FC	DWF	QPCR	Reference (MF)	Reference (CS)
Offshore Seawater w/Sewage(1)	1	220	881	67	40	26
Offshore Seawater w/Sewage(2)	1	422	773	86	73	37
Offshore Seawater w/Sewage(3)	0	596	1,113	927	661	512

MF – Membrane filtration
CS – Chromogenic substrate

cleavage of the chromogenic substrate in these samples. This is perplexing, as DFW employs the same fluorogenic substrate, 4-methylumbelliferyl-β-D-glucoside, as used in the Enterolert® method and none of the reference laboratories using this method exhibited a similar trend for the same samples. This suggests a low-level artificial hydrolyzation of the substrate by constituents of the matrix that occurred early in the incubation and was detectable by the advanced optics of DFW, but kinetic-limited and nondetectable by the naked eye following the typical extended Enterolert® incubation. Further work will be necessary to test this hypothesis, as urban runoff contamination is an important concern that motivates beach water quality monitoring in southern California.

The FC method registered high values (ca. 1000 cells/100 mL) for almost all samples, including most blanks. This is particularly problematic, as it does not provide for discrimination between contaminated and non-contaminated sites. The overestimates could arise at several places in the measurement process, including attachment of the antibodies to non-target organisms or incorrect identification of non-cellular material, such as suspended solids, by the flow cytometer. As similar antibodies used in this work have been found to be effective in other applications, the more likely difficulty is with quantification by the flow cytometer. Flow cytometers have a long history of successful quantification of microbial cells in other applications, but many of these applications involve quantification of all cells in the fluid and differentiation by parameters such as size or natural fluorescence, rather than differentiation between similarly sized cells tagged with an immunological marker (Campbell 2001). Further work to differentiate between target and non-target cells in this counting process is probably necessary for this method to succeed.

The IMS/ATP method had the opposite problem, measuring values near zero for most samples. This could be due to poor antibody recognition of the target, but that is unlikely because this method has produced results comparable to existing methods in previous freshwater testing (Lee and Deininger 2004) and in informal participation in previous comparative testing with California marine samples (Griffith *et al.* 2004). A more likely explanation is failure of the magnetic system used to capture bacteria after antibody attachment. The magnetic capture system was substantially redesigned prior to this test and its poor performance could explain the universally low values recorded. Low values in all samples could also result from failures in the ATP quantification system. While this method has been shown to work in previous trials (Deininger and Lee 2001), poor quality reagents or incorrect calibration of the equipment in this particular implementation could have occurred. Further research is needed to assess success at each of the stages in the capture and measurement system.

While none of the new rapid methods produced results equivalent to those of the reference laboratories, all of the methods were transferred easily to local personnel with minimal training investment. All of the local participants indicated a comfort level in implementing the methods. Moreover, the precision of their results was comparable to that when the method developers performed the methods. While the local practitioners generally were laboratory managers and had more experience than the typical technician, many had no specific previous training in many of the areas employed here, such as micropipetting or PCR. While there is need for performance improvement in each of the new methods, technology transfer does not seem to be an important impediment to method adoption.

Literature Cited

Alves, P.I., M.P. Martins, T, Semedo, J.J. Figueiredo Marques, R. Tenreiro and M.T. Barreto Crespo. 2004. Comparison of phenotypic and genotypic taxonomic methods for the identification of dairy enterococci. *Antonie Van Leeuwenhoek* 85: 237-52.

Boehm, A.B., S.B. Grant, J.H. Kim, S.L. Mowbray, C.D. McGee, C.D. Clark, D.M. Foley and D.E. Wellman. 2002. Decadal and shorter period variability and surf zone water quality at Huntington Beach, California. *Environmental Science and Technology* 36: 3885-3892.

Brinkman, N.E., R.A. Haugland, L.J. Wymer, B. Murulrrdhara, R.L. Whitman and S.J. Vesper. 2003. Evaluation of a rapid, quantitative real-time PCR method for enumeration of pathogenic *Candida* cells in water. *Applied and Environmental Microbiology* 69: 1775-1782.

Campbell, L. 2001. Flow cytometric analysis of autotrophic picoplankton. pp. 317-343 *in:* J.H. Paul (ed.), Methods in Microbiology: Marine Microbial Ecology 30. Academic Press.

Deininger, R.A. and J.Y. Lee. 2001. Rapid Determination of Bacteria in Drinking Water Using an ATP Assay. *Field Analytical Chemistry and Technology* 5: 185-189.

Domig, K.J., H.K. Mayer and W. Kneifel. 2003. Methods used for the isolation, enumeration, characterisation and identification of *Enterococcus spp*. 2. Pheno- and genotypic criteria. *International Journal of Food Microbiology* 88:165-88.

Dupray, E., M.P. Caprais, A. Derrien and P. Fach. 1997. Salmonella DNA persistence in natural seawaters using PCR analysis. *Journal of Applied Microbiology* 82: 507-10.

Fung, D.Y.C. 2002. Rapid methods and automation in microbiology. *Comprehensive Reviews in Food Science and Food Safety* 1: 1-20.

Griffith, J.F., L.A. Aumand, I.M. Lee, C.D. McGee, L.L. Othman, K.J. Ritter, K.O. Walker and S.B. Weisberg. 2004. Comparison and verification of bacterial water quality indicator measurement methods using ambient coastal water samples. This volume.

Harwood, V.J., N.C. Delahoya, R.M. Ulrich, M.F. Kramer, J.E. Whitlock, J.R. Garey and D.V. Lim. 2004. Molecular confirmation of *Enterococcus faecalis* and *E. faecium* from clinical, faecal and environmental sources. *Letters in Applied Microbiology* 38: 476-82.

Haugland, R. A., N.L. Brinkman and S.J. Vesper. 2002. Evaluation of rapid DNA extraction methods for the quantitative detection of fungi using real-time PCR analysis. *Journal of Microbiological Methods* 50: 319-323.

Haugland, R. A., J. L. Heckman and L.J. Wymer. 1999. Evaluation of different methods for the extraction of DNA from fungal conidia by quantitative competitive PCR analysis. *Journal of Microbiological Methods* 37: 165-176.

Lee, J.Y. and R. A. Deininger. 2004. Detection of *E. coli* in beach water within 1 hour using immunomagnetic separation and ATP bioluminescence. *Luminescence* 19: 31-36.

Leecaster, M.K. and S.B. Weisberg. 2001. Effect of sampling frequency on shoreline microbiology assessments. *Marine Pollution Bulletin* 42: 1150-1154.

Ludwig, W. and K.H. Schleifer. 1994. Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiol. Reviews* 15: 155-173

Noble, R.T., M. Leecaster, C. D. McGee, S.B. Weisberg and K. Ritter. 2004. Comparison of bacterial indicator analysis methods in stormwater-affected coastal waters. *Water Research* 38: 1183-1188.

Noble, R.T., D.F. Moore, M. Leecaster, C.D. McGee and S.B. Weisberg. 2003. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research* 37: 1637-1643.

Noble, R.T. and S.B. Weisberg. 2004. A review of technologies being developed for rapid detection of bacteria in recreational waters. *Journal of Water and Health.* In Press.

Noble, R.T., S.B. Weisberg, M.K. Leecaster, C.D. McGee, K. Ritter, K.O. Walker and P.M. Vainik. 2003. Comparison of beach bacterial water quality indicator measurement methods. *Environmental Monitoring and Assessment* 81: 301-312.

NRC (National Research Council). 2004. Indicators for waterborne pathogens. National Academies Press. Washington, DC.

Sambrook, J., D.W. Russell and J. Sambrook. 2001. Molecular Cloning: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.

Santo Domingo, J.W., S.C. Siefring and R.A. Haugland. 2003. Real-time PCR method to detect Enterococcus faecalis in water. *Biotechnology Letters* 25: 261-5.

Simmon, K.E., D.D. Steadman, S. Durkin, A. Baldwin, W.H. Jeffrey, P. Sheridan, R. Horton and M.S. Shields. 2004. Autoclave method for rapid preparation of bacterial PCR-template DNA. *Journal of Microbiological Methods* 56: 143-9.

Tippett, J.M., K. Harrigan, L.M. Erdahl and K.R. Harkins. 2002. A rapid antibody specific method for the detection of food pathogens from environmental surfaces. Poster presentation, International Association of Food Protection Meeting. San Diego, CA

Velasco, D., S. Perez, F. Pena, M.A. Dominguez, M. Cartelle, F. Molina, R. Moure, R. Villanueva and G. Bou. 2004. Lack of correlation between phenotypic techniques and PCR-based genotypic methods for identification of Enterococcus spp. *Diagn Microbiol Infect Dis.* 49: 151-6.

Wilson, I.G. 1997. Inhibition and facilitation of nucleic acid amplification. *Applied and Environmental Microbiology* 63: 3741-3751

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