

ORIGINAL ARTICLE

Covalently linked immunomagnetic separation/adenosine triphosphate technique (Cov-IMS/ATP) enables rapid, in-field detection and quantification of *Escherichia coli* and *Enterococcus* spp. in freshwater and marine environments

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Keywords

environmental/recreational water, indicators, rapid methods, water, water quality.

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2009/1908: received 3 November 2009,
revised 15 December 2009, accepted 16
December 2009

doi:10.1111/j.1365-2672.2009.04660.x

Abstract

Aims: Developing a rapid method for detection of faecal pollution is among the critical goals set forth by the Environmental Protection Agency in its revision of water quality criteria. The purpose of this study is to devise and test covalently linked antibody–bead complexes for faecal indicator bacteria (FIB), specifically *Escherichia coli* or *Enterococcus* spp., in measuring water quality in freshwater and marine systems.

Methods and Results: Covalently linked complexes were 58–89% more robust than antibody–bead complexes used in previous studies. Freshwater and marine water samples analysed using covalently linked immunomagnetic separation/adenosine triphosphate quantification technique (Cov-IMS/ATP) and culture-based methods yielded good correlations for *E. coli* ($R = 0.87$) and *Enterococcus* spp. ($R = 0.94$), with method detection limits below EPA recreational water quality health standards for single standard exceedances (*E. coli* – 38 cells per 100 ml; *Enterococcus* spp. – 25 cells per 100 ml). Cov-IMS/ATP correctly classified 87% of *E. coli* and 94% of *Enterococcus* spp. samples based on these water quality standards. Cov-IMS/ATP was also used as a field method to rapidly distinguish differential loading of *E. coli* between two stream channels to their confluence.

Conclusions: Cov-IMS/ATP is a robust, in-field detection method for determining water quality of both fresh and marine water systems as well as differential loading of FIB from two converging channels.

Significance and Impact of the Study: To our knowledge, this is the first work to present a viable rapid, in-field assay for measuring FIB concentrations in marine water environments. Cov-IMS/ATP is a potential alternative detection method, particularly in areas with limited laboratory support and resources, because of its increased economy and portability.

Introduction

Rapid detection methods and alternative indicators are at the forefront of developing initiatives to ensure clean and safe water quality (USEPA 2007). Pollution from diverse and multiple sources have historically confounded efforts to preserve coastal and freshwater environments. The devel-

opment of rapid detection assays, allowing enumeration of microbial contaminants as quickly as 1 h and enabling a more diverse suite of organisms to be studied, has progressed significantly in recent years (Griffith and Weisberg 2006). Among these explored technologies are quantitative polymerase chain reaction (qPCR) (Haugland *et al.* 2005; Caldwell *et al.* 2007; Shanks *et al.* 2008), antibody

fluorescence (Garcia-Armisen *et al.* 2005; Zimmerman *et al.* 2009), enzymatic methods (Lebaron *et al.* 2005), flow cytometry (Caruso *et al.* 2008; Hammes *et al.* 2008) and immunomagnetic separation (IMS)/ATP quantification (Lee and Deininger 2004; Bushon *et al.* 2009a). IMS/ATP is particularly advantageous because it is field portable (eliminating travel time), the most rapid of the mentioned processes (<1 h processing time), and viability based.

IMS has been used as a selection step in measuring protocols for *Giardia* (USEPA 2005; Hsu and Huang 2007), *Cryptosporidium parvum* (Rochelle *et al.* 1999) and *E. coli* O157 (Tomoyasu 1998). Complexes, comprised of antibodies sorbed to magnetic beads, are used to capture and separate out a target population with the application of a magnetic field. After targets are isolated, cells are ruptured and ATP is quantified through the addition of luciferin/luciferase. Luciferase, in the presence of luciferin and oxygen, catalyses a reaction that consumes ATP and emits light as a by-product. Intensity of this light emission is measured by a luminometer and correlated to cell concentration. IMS/ATP has been the basis for quantifying pathogenic or bacterial activity and/or contamination in numerous applications such as in the food industry (Siragusa *et al.* 1996; Tu *et al.* 2000; Murphy *et al.* 2007) in veterinary applications (Watarai *et al.* 2005), in drinking water (Deininger and Lee 2001; Delahaye *et al.* 2003) and in wastewater (Allegra *et al.* 2008; Bushon *et al.* 2009b). Although an IMS/ATP-based assay has numerous advantages (portable, specific, and economical), this method has been relatively understudied.

Recently, significant progress has been made to translate the applicability of IMS/ATP to recreational water quality monitoring; notably, in 2004, Lee and Deininger were the first to publish such findings, which describe the development of this assay for monitoring *E. coli* in recreational freshwater environments. Bushon *et al.* were able to adapt this work further, optimizing Lee and Deininger's protocol to measure *Enterococcus* spp. in freshwater (Bushon *et al.* 2009a) and both *E. coli* and *Enterococcus* spp. in wastewater influent (Bushon *et al.* 2009b). The selective magnetic bead-antibody complex used to isolate target organisms out of an environmental sample utilizes hydrophobic sorption as the primary attachment mechanism. This complex, however, can be destabilized fairly easily, even with physical (pipetting/vortexing) or chemical (use of a nonionic detergent) treatment, which may explain why the method has not been validated in a marine system.

The presented work achieves three significant steps towards using an IMS/ATP-based assay in recreational water quality monitoring. We (i) developed a more robust antibody-bead complex that is covalently linked. When this complex is coupled with ATP quantification [covalently linked-IMS/ATP (Cov-IMS/ATP)], we show

that it (ii) can be used for marine and fresh water quality analysis for *E. coli* and *Enterococcus* spp. and (iii) can be instrumented as a field-portable system that adaptively tracks faecal indicator bacteria (FIB) in a local impaired watershed. These combined improvements are also examined in the context of a more efficient and effective tiered source-tracking strategy.

Materials and methods

Antibody-Dynabead biosorbent

Antibodies specific to each FIB were used to generate biosorbents that targeted *E. coli* (Cat#B65001R) or *Enterococcus* spp. (Cat#B65173R, ent) (Meridian Life Sciences 2009a,b). *Escherichia coli* antibodies are selective to *E. coli* with an O or K antigen, which represent for a broad range of *E. coli* serotypes (Ørskov *et al.* 1977) be selected for in the environment. The manufacturer datasheet reported potential cross-reactivity with *Enterobacteriaceae* such as *Shigella* and *Salmonella*. This cross-reactivity was tested by Lee and Deininger (2004) and on average accounted for *c.* 10% of the bound population and, with respect to *Enterococcus* spp. antibodies, minimal cross-reactivity was detected in a similarly constructed polyclonal IgG by Caruso *et al.* (2008). For these reasons, specificity was not independently explored in this work.

Dynabead particles (142.04, M-280; Invitrogen, Carlsbad, CA) are uniform, superparamagnetic, polystyrene beads coated with a polyurethane layer. Dynabeads are functionalized with sulfonfyl ester surface groups that permit chemical covalent attachment (amino or sulfhydryl) of ligand proteins such as immunoglobulins (IgG). The typical particle diameter is 2.8 μm and has an average density of 1.4 g cm^{-3} . The hydroxy groups on the Dynabeads are activated using p-toluensulfonyl chloride.

Coupling protocol

We washed 350 μl Dynabeads in borate buffer (0.1 mol l^{-1} , pH 9.5) and separated them from solution using a rare earth magnet. After the second wash, clean Dynabeads were added to the 55 μl IgG solution. The final suspension was incubated at 37°C on a rotating mixer for 16–24 h. The final mixture was washed and stored in bovine serum albumin buffer at continuous rotation at 4°C.

Adsorption-based IMS/ATP protocol was obtained from previous works (Lee and Deininger 2004; Bushon *et al.* 2009a). Robustness of the covalently coupled antibody-bead complexes presented in this work was compared to that of adsorption-based antibody-bead complexes. This was accomplished by measuring the integrity of the attachment that was sustained after two mixing treatments:

pipette-mixing and vortex-mixing, each for 10 s and for two washes. Uncoupled or detached IgG in the supernatant was measured using a bicinchoninic acid (BCA) protein assay kit (Cat# 23225; Pierce, Rockford, IL).

Culture-based methods (IDEXX (Westbook, MN) and EPA Methods 1600/1603)

Standard methods of measuring FIB concentrations were IDEXX and membrane filtration. These protocols were used to calibrate Cov-IMS/ATP to standard units; IDEXX Quanti-Tray and Colilert-18 (*E. coli*) or Enterolert (enterococci) yielded values of *E. coli* or enterococci concentrations in most probable number (MPN) per 100 ml in freshwater. EPA membrane filtration methods 1600 and 1603 were used for marine sample measurements of enterococci (USEPA 2006a) and *E. coli* (USEPA 2006b), respectively, and yielded values in colony forming units (CFU) per 100 ml. Both IDEXX and membrane filtration methods were used, because there is considerable interference when using IDEXX to enumerate marine sample concentrations (Pisciotta *et al.* 2002; Griffith *et al.* 2006) and were conducted on the resuspension and converted to per 100 ml original sample volume for analysis. Previous works have shown that IDEXX and membrane filtration methods are comparable (Noble *et al.* 2003a,b, 2004).

Cov-IMS/ATP target ATP assay

Samples were incubated with anti-*E. coli* or anti-ent biosorbent for 30 min at ambient temperature. A magnetic separator was used to separate biosorbent with bound targets from remaining solution. The bound complexes were washed with phosphate buffered saline (PBS) followed by 200 μ l of somatic cell-releasing agent to eliminate materials that could cause interference. The bound product was lysed using 50 μ l bacteria cell-releasing agent and treated with 50 μ l luciferin/luciferase to initiate the light-emitting reaction. A luminometer (model 3550; New Horizons Diagnostics) was used to quantify the intensity of emitted light. A minimum of three measurements was collected per sample, consisting of a 30-s integration average for each value of light intensity in relative light units (RLU). Processing time of each sample was *c.* 36 min each, as numerous samples were processed concurrently.

Field application

Several field-adapted components were integrated into the assay protocol to facilitate on-site processing. A rapidly deployable field station consisted of a portable lab bench (Coleman Model 2790-490; Coleman, Wichita, KS), deep-cycling battery, AC/DC inverter, Millipore vac-

uum/pressure pump (WP6111560; Millipore, Billerica, MA), cordless power drill (DC987K2; De Walt, Baltimore, MD), Rotomix Orbital Shaker (12-815-2D; Fisher Scientific, Pittsburgh, PA), filtration apparatus, several small coolers, luminometer and general supplies. Antibody-bead complexes were kept in continuous suspension manually or using the shaker. Mixing was achieved through repeated pipetting. Constant rotation was achieved by using a battery-powered drill and, while revolutions per minute may have varied somewhat (55–65 rev min⁻¹), care was taken to ensure that bead-containing tubes were being gently mixed. All equipment was able to be packaged into a Pelican case (Model 1620; Pelican Products, Torrance, CA) and transported in a standard hatchback vehicle or four-door sedan.

Field sampling

Freshwater site (Fig. 1)

Will Rogers State Beach (WRSB) is located in Santa Monica, CA. Two urbanized channels – West Channel and Entrada, drain into WRSB through the Santa Monica Canyon channel system. During dry season (April–October), a low-flow diversion is used to help mitigate impact to the beach by diverting channel flow to a wastewater treatment plant. Potential sources of FIB include significant bird populations, algae lining the channel and storm drains (0.3–1.2-m diameter openings) with dry and wet weather runoff. There is often a pool of standing water at the WRSB channel outlet.

Samples were collected on multiple field days during August 2008–February 2009 from eleven sites in the Santa Monica Canyon channel system; six sites from Entrada (E1–E6), four from West Channel (W1–W4) and one from the confluence (C1), were sampled at different times throughout the day. A varying subset of the 11 total collected on each field day were analysed using both Cov-IMS/ATP and standard culture-based methods.

Coastal survey

Anti-*E. coli* or anti-ent biosorbents were tested in marine water samples from beaches in Santa Monica Bay and/or Orange County. Field sites were determined by evaluating historical FIB data obtained from Heal the Bay (<http://www.healthebay.org>). Several beaches (denoted with an *) were on average in exceedance of the health standard (single sample standards at 104 MPN 100 ml⁻¹ for enterococci and 400 MPN 100 ml⁻¹ for *E. coli*) for FIB (USEPA 1986a). The sampled sites included *Puerco Beach, Surf rider Beach, Topanga Canyon Beach, *Dockweiler Beach, *Santa Monica Beach at the pier and Doheny State Beach in South Orange County during the SCCWRP epidemiological study in summer 2008.

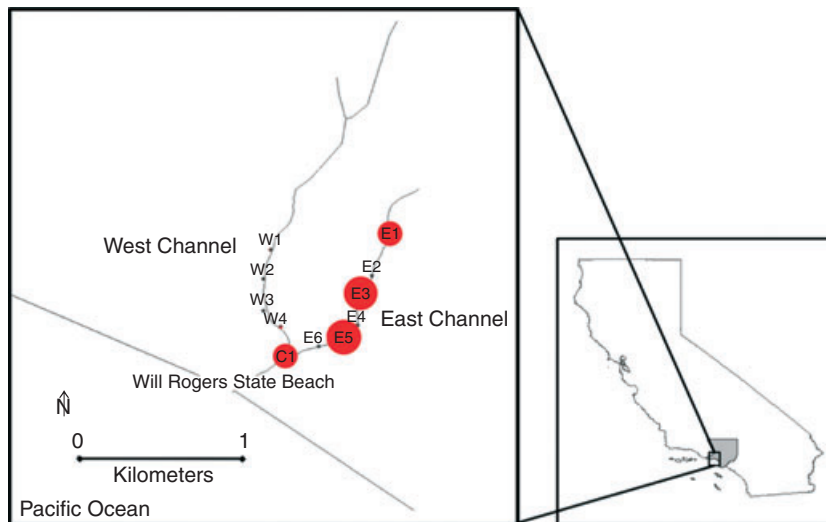


Figure 1 Map of sampling locations in the Santa Monica Canyon Channel with relative concentrations of *Escherichia coli*.

Sample processing

Approximately 500–1000 ml per sample was filtered-concentrated and analysed using Cov-IMS/ATP and culture-based methods. Samples were filtered once through a 20- μm pore size filter (NY2004700; Millipore) to remove large particles and debris followed by a 0.45- μm filter (SA1J792H5; Millipore) to capture bacteria. Bacteria were extracted into 5–10 ml PBS from the 0.45- μm filter through resuspension in phosphate buffer solution (0.1 mol l^{-1} , pH 7.4) by manual shaking or on a vortex (lab) for 1 min. For a subset of samples, the 20- μm filter was enumerated using membrane filtration method to account for organisms that could possibly be attached to the surfaces of trapped debris. One to 1.5 ml of resuspension was added to the anti-*E. coli* or anti-ent biosorbent, incubated on a rotating mixer for 30 min at ambient temperature and processed according to methods described previously. Remaining resuspension was analysed using IDEXX or membrane filtration for *E. coli* or enterococci, respectively. Field blanks were prepared by sterilizing samples from the field site and processed after filtering and resuspending in PBS while lab blanks consisted of measuring ATP in sterilized PBS or milli-Q water.

Luminometer calibration

The luminometer was calibrated using standard ATP solution (FLAAS-5VL; Sigma Aldrich, St Louis, MO) diluted to 0.22–43 $\mu\text{mol l}^{-1}$ (actual mol amount measured ranged from 11 to 215 pmol in 50 μl) to account for shift in signal because of inherent variability of the instrument.

Effect of hold time

One sample at site E5 was taken at c. 10:00 AM; 500 ml of sample was filtered and resuspended into 10 ml of

PBS. One millilitre of resuspension was measured after 0 min, 15 min, 2 h and 6 h of incubation on ice to determine the effect of holding time on ice on of target cell ATP.

Statistics

Spearman rank correlation coefficients were calculated in Excel. SPSS statistical software (SPSS Inc., Chicago, IL) was used to determine correlation coefficients as well as *P*-values for the relationship between culture-based method results and Cov-IMS/ATP results.

Results

Covalent (COV) and hydrophobic adsorption (ADS) attachment and antigen-capture efficiency

Attachment efficiency between the IgG antibodies and magnetic beads were comparable between COV and ADS-based complexes (c. 80% attachment, when comparing concentrations of antibody prior to and post coupling). Retention of antibodies to the magnetic beads after undergoing vortexing or pipetting treatments were also compared by measuring IgG concentrations in the supernatant of the respective complexes (Fig. 2). For ADS complexes, the complex retained 41.6% (pipette-mixing) and 11% (vortexing) of the antibodies that attached whereas there was no detectable loss when COV-biosorbents underwent the same treatment. Percent retention for COV-biosorbents was calculated based on one half the detection limit of the ligand assay used to measure antibody concentrations, which corresponded with a 1.1% loss of antibody. The percent loss values were averaged from duplicates. On a separate set of complexes (that did not undergo physical treatments, only sample pro-

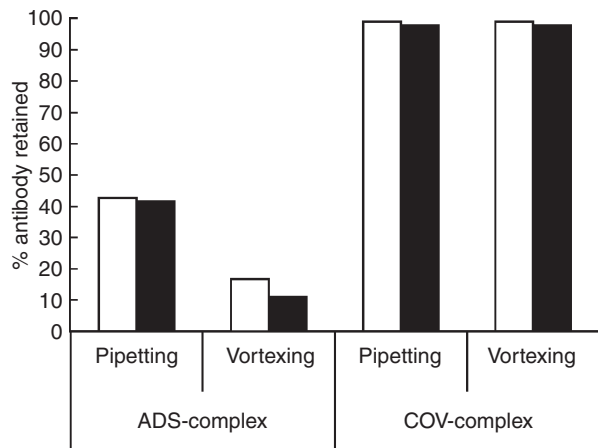


Figure 2 Physical treatments (mixing via pipetting or vortexing) destabilizes the antibody–bead complex that is generated through hydrophobic sorption. (□) Postwash 1 and (■) Postwash 1 + 2.

cessing), *E. coli* concentrations were also measured using IDEXX prior to and after incubation; COV-beads bound more than twice as many *E. coli* cells as ADS-beads; 67% (COV) vs 31% (ADS) of the added *E. coli* population were measured by mass balance to have attached to the antibodies ($(E. coli_{added} - E. coli_{supernatant}) / (E. coli_{added})$).

Effect of hold time on sample ATP

The amount of measured ATP per culturable *Enterococcus* spp. cell decreased when the sample resuspension was held on ice. Solution temperature was also recorded each time a sample was analysed. Results (Fig. 3) reveal that target cellular ATP measurements decreased by 26% within 15 min of being stored on ice and 30% after 2 h. At 6 h, a decrease of 38% from the 0 time point of sample ATP values was observed.

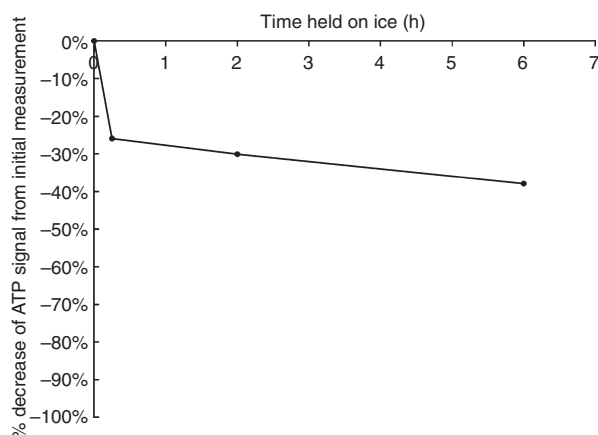


Figure 3 Decrease in relative light units signal as a function of hold time on ice.

***Escherichia coli* and *Enterococcus* spp. calibration between Cov-IMS/ATP and traditional methods (Figs 4 and 5, respectively)**

Freshwater samples were measured using Cov-IMS/ATP and IDEXX while marine samples were enumerated with Cov-IMS/ATP and membrane filtration (marine samples analysed using IDEXX are marked as open triangles). Both analyses were conducted on filtered resuspension of the sample; plotted values were adjusted to units of 100 ml original sample using amount of volume filtered and quantified using each respective method. The calibration relationships are summarized as follows:

$$E. coli: \text{Log RLU } 100 \text{ ml}^{-1} = 0.66 \text{Log MPN or CFU } 100 \text{ ml}^{-1} + 3.48, R = 0.87 \tag{1a}$$

$$Enterococcus \text{ spp.}: \text{Log RLU } 100 \text{ ml}^{-1} = 0.69 \times \text{Log MPN or CFU } 100 \text{ ml}^{-1} + 3.93, R = 0.94 \tag{1b}$$

Cov-IMS/ATP (RLU per 100 ml) correlates well with both *E. coli* and *Enterococcus* spp. in both environmental water samples ($R = 0.87$ and 0.94 , respectively). The lower detection limit of *E. coli* based on the combined dataset of freshwater and marine samples was *c.* 38 cells per 100 ml sample and the calculated limit for *Enterococcus* spp. is 25 cells per 100 ml. These detection limits were determined using the method detection limit document generated by the EPA (USEPA 1986b). Calibration of instrument with ATP standards was conducted frequently to ensure consistency of measurements by luminometer.

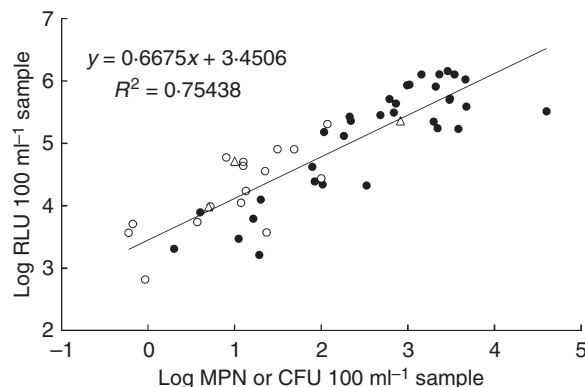


Figure 4 *Escherichia coli* correlation between culture-based methods (IDEXX or membrane filtration, MPN or CFU 100 ml⁻¹ sample) and Cov-IMS/ATP (relative light units – RLU 100 ml⁻¹ sample). $R = 0.87$. (○ = Marine samples MF; △ = Marine samples IDEXX; ● = Freshwater samples IDEXX).

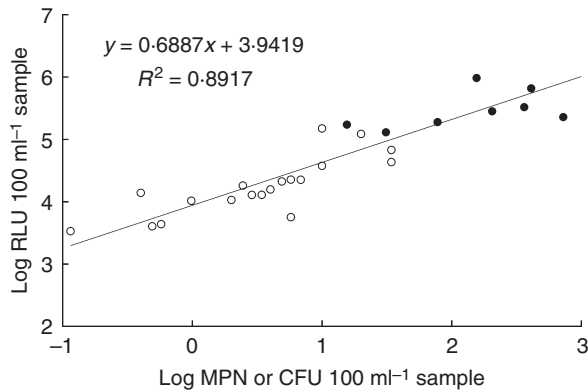


Figure 5 *Enterococcus* spp. correlation between culture-based methods (IDEXX or membrane filtration, MPN or CFU 100 ml⁻¹ sample) and Cov-IMS/ATP (relative light units – RLU 100 ml⁻¹ sample). $R = 0.94$. (○ = Marine samples IDEXX; ● = Freshwater samples).

Water quality classification (Figs 6 and 7)

We used eqn (1a,b) to evaluate how consistently Cov-IMS/ATP values predicted exceedances and nonexceedances in water quality standards. Correct exceedance (Quadrant II) and correct nonexceedance predictions (Quadrant III) are specified by the points in the upper right and lower left quadrants of the plot, respectively. False positives (Quadrant I) occurred when Cov-IMS/ATP correlation indicated a CFU or MPN 100 ml⁻¹ value that exceeded the health standard when culture-based methods did not, and false negatives (Quadrant IV) occurred when Cov-IMS/ATP indicated a CFU or MPN 100 ml⁻¹ that did not exceed the health standard when culture-based methods did. The percentage of correct classification ranged from 87 and 94 for *E. coli* ($n = 52$) and *Enterococcus* spp. ($n = 29$), respectively. The solid and dashed lines represent the intersection of sam-

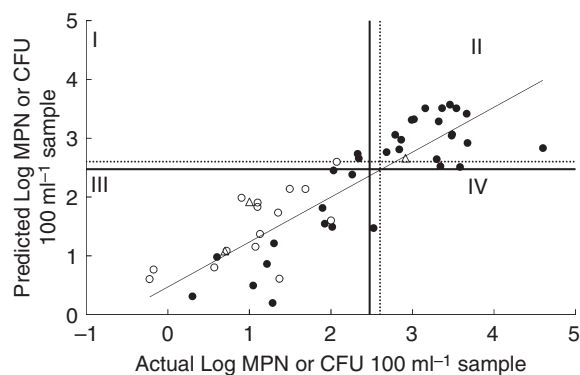


Figure 6 *Escherichia coli* concentrations, as predicted by Cov-IMS/ATP, have a 92% correct classification rate ($n = 52$). (○ = Marine samples IDEXX; ● = Freshwater samples).

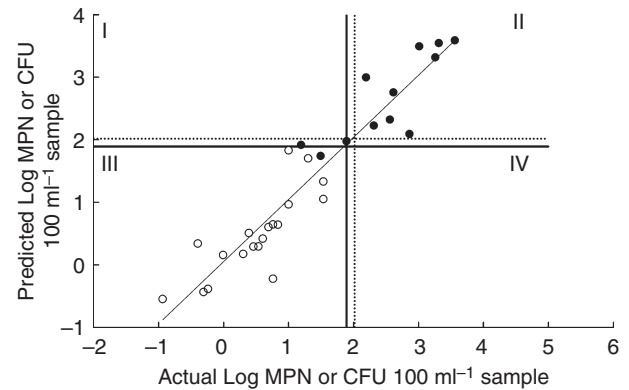


Figure 7 *Enterococcus* spp. concentrations, as predicted by Cov-IMS/ATP, have a 94% correct classification rate ($n = 31$). (○ = Marine samples IDEXX; ● = Freshwater samples).

ple exceedance with respect to the single standard in freshwater and marine recreational waters, respectively (USEPA 1986a).

Filter recovery and analysis

There were no colonies on the enumerated 20- μ m filters that were used for prefiltration, although solids and particulates were visibly collected and separated from the volume that underwent the 0.45- μ m filtration. Recovery (comparing the resuspension to the original sample) was determined for ten samples and ranged from 60 to 85%. Several low-concentration samples appeared to have a >100% recovery efficiency, although this may be accounted for because of increased detection limit of concentrating a larger volume. It is important to note that while filter-concentration can still be further optimized, it significantly improves the detection limit of a method.

Cov-IMS/ATP as a tool for adaptive sampling

When this method used in-field, we were able to determine differences in the contributing loads at the channels confluence: the *E. coli* concentration at Entrada-E6 was *c.* 20-fold greater than that of West Channel-W4 (Fig. 1). When this model was applied retrospectively to remainder of samples on that date, there were significantly higher inputs of *E. coli* from Entrada than there are along West Channel. A more detailed view of Entrada inputs (Fig. 3) revealed that the concentration of *E. coli* increased nearly 1.4 times between E1 and E3 (from 921 to 1254 MPN⁻¹100 ml⁻¹) and increased slightly (*c.* 4%) at E5 (from 1254 to 1300 MPN 100 ml⁻¹) before reaching the confluence C1 (at 913 MPN 100 ml⁻¹).

Another potential application for rapid results was employed with FIB results from Santa Monica Canyon Channel; W4, E5 and C1 were applied to a weighted average mixing equation as conservative tracers. Flow calculations were based on measurements taken on the channel in-field (depth at multiple points along the flow, width of the flow stream, velocity), which were highly variable because of the heterogeneities in surface roughness and the presence of debris. Both assays (IDEXX and Cov-IMS/ATP) predicted C1 values that were within close range at +14 and -28%, respectively, of actual values as measured by each technique.

Statistical analysis and reproducibility

The Spearman rank correlation coefficients were calculated for log-transformed values of RLU and MPN or CFU and significantly correlated for *E. coli* ($\rho = 0.94$) and *Enterococcus* spp. ($\rho = 0.95$). Spearman correlations were conducted on all samples, including blanks. These values were considered at a significance level of 0.01 for the respective sample set. Regression coefficients also reflected a good fit to the data for *E. coli* ($R = 0.87$) and *Enterococcus* spp. in ($R = 0.94$) with P -values < 0.001 for both *E. coli* and *Enterococcus* spp. Reproducibility was also examined in controlled lab experiments, in which four samples replicates were conducted. Samples were comprised of a diluted lab culture (*E. coli*, ATCC#12014) and were processed according to the target ATP assay. The sample values exhibited a 6.8% error (ratio of standard deviation to mean) for four samples. Additional field samples and duplicates reflected a range of variability from 6 to 25%.

Discussion

COV-biosorbents and ADS-biosorbents robustness

Cov-IMS/ATP has an added robustness over previous antibody-bead complexes employed in recreational water quality measurements with IMS/ATP (Lee and Deininger 2004; Bushon *et al.* 2009a,b); because antibodies are covalently linked to paramagnetic beads, they are less likely to disengage during sample processing than adsorption-based complexes are. More specifically, we observed a minimal loss of antibody attachment in COV-beads ($< 1.1\%$) in comparison with ADS-beads (89% for vortex-mixing and 57.5% for pipette-mixing). Previous documentation is consistent with this finding, whether the complexes are undergoing physical (Bangs Laboratory 1999, 2002a,b) or chemical (Meisenberg and Simmons 2006; Bushon *et al.* 2009a,b) treatments. It is possible that this detachment contributed to the high percentage of

false negatives observed in previous work (Griffith *et al.* 2004) as well as the poorly correlated measurements between IMS/ATP and culture-based methods, likely because of the presence of interfering materials, from one of the wastewater treatment sites reported by Bushon *et al.* (2009b).

Variability and use of Cov-IMS/ATP technique as classification tool

One reason for apparent overestimation of cells (false positives) with Cov-IMS/ATP compared to culture-based methods may be nonspecific binding that results in the capture and quantification of organisms that are cross-reacting with the antibody. Another possible factor is that Cov-IMS/ATP is capable of measuring viable but nonculturable cells, which are not accounted for in membrane filtration or IDEXX. Recent work with the use of antibodies has shown that they can be used to visualize both culturable and nonculturable target organisms (Zimmerman *et al.* 2009). Furthermore, Cov-IMS/ATP can potentially be used for monitoring in marine waters, which is a significant addition to the capacity of previous IMS/ATP assays. Validation in marine samples may be largely because of the added robustness of the biosorbent as well as the filtered resuspension into phosphate buffer; filtering improves the detection limit and allows quantification of targets even in low concentrations in marine water.

Adsorption-based IMS/ATP has recently expanded to measurement of enterococci in freshwater (Bushon *et al.* 2009a) and FIB in wastewater samples (Bushon *et al.* 2009b), and this work has shown that Cov-IMS/ATP can be applied in marine systems for *E. coli* and *Enterococcus* spp. *Enterococcus* spp. has recently become the preferred indicator (with regard to standard FIB) for marine sites (Noble *et al.* 2003a,b), although *E. coli* is still relevant in freshwater (Kinzelman *et al.* 2003) and can still be useful in marine systems receiving significant, urban-impacted freshwater inputs (Noble *et al.* 2006; He and He 2008).

In-field processing improves efficacy in the tiered approach through rapid, adaptive sampling

A rapid, portable method has been identified as an important tier to the multitiered approach to source-tracking (Field and Samadpour 2007) which has conventionally relied on day-old culture-based results (Boehm *et al.* 2003; Noble *et al.* 2006) or on hydrologic events as the intermediate tier. The inefficiencies of traditional tiered approach may arise from the temporal variability of FIB in aquatic environments (Boehm *et al.* 2002; Boehm and Weisberg 2005; Boehm 2007) as well as

differences in the survival behaviour of FIB and the pathogens they proxy (Geldenhuys and Pretorius 1989; Lund 1996; Lemarchand and Lebaron 2003; Harwood *et al.* 2005). After determining that a higher *E. coli* input was originating from Entrada than at West Channel, we are able to visualize load distribution throughout Santa Monica Channel (Fig. 1). This map, which can be generated on-site, can provide important, near-real time insight into where additional samples should be collected. This adaptive sampling approach may significantly improve the efficiency of a tiered method, especially as Cov-IMS/ATP is further streamlined and field-packaged, and assays for additional targets are developed.

Processing a sample on-site also minimizes differences in ATP concentration arising from varying sample exposure to ice. Hold time could possibly be controlled and optimized for, depending on the target organisms' varying response to temperature and ATP synthesis/degradation. Previous work has shown that ATP concentrations are stable when cells are at steady-state (Schneider and Gourse 2004); in this context, holding on ice may likely impact the kinetics between the antibody and the target rather than internal ATP values. Observed variation in environmental sample duplicates could also be the result of keeping samples on ice. Cov-IMS/ATP is a good alternative to other proposed methods for laboratories and agencies that do not have the resources to support a full-scale laboratory.

Conclusions

Cov-IMS/ATP presents several improvements and assets to water quality monitoring over previous works and approaches: (i) the Cov-IMS/ATP biosorbent is more robust than previous biosorbents that were constructed through hydrophobic sorption; (ii) this is the first work to present a Cov-IMS/ATP assay for marine water applications and (iii) through field-portability and rapid detection, Cov-IMS/ATP can significantly improve the efficacy of the tiered approach to source-tracking because it can be used to adaptively sample and identify hotspots of faecal contamination inputs.

Acknowledgements

We thank Katie Mika, David Ginsburg, Vanessa Thulsiraj and SCCWRP for field and laboratory support. This work was supported the University California Center for Water Resources and the National Science Foundation (Grant ANI-00331481 for Networked Info Mechanical Systems (NIMS)). We would also like to thank anonymous reviewers for their suggestions which helped us improve our manuscript.

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