WATER RESEARCH XXX (2009) 1–7

Available at www.sciencedirect.com

journal homepage: www.elsevier.com/locate/watres

Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater

Rebecca N. Bushon[*], *Christina A. Likirdopulos*[1], *Amie M.G. Brady*

US Geological Survey, 6480 Doubletree Avenue, Columbus, OH 43229-1111, USA

ARTICLE INFO

Article history:
Received 14 March 2009
Received in revised form
16 June 2009
Accepted 22 June 2009
Published online ■

Keywords:
E. coli
Enterococci
Wastewater
Rapid method
Immunomagnetic separation
ATP detection

ABSTRACT

Untreated wastewater samples from California, North Carolina, and Ohio were analyzed by the immunomagnetic separation/adenosine triphosphate (IMS/ATP) method and the traditional culture-based method for *E. coli* and enterococci concentrations. The IMS/ATP method concentrates target bacteria by immunomagnetic separation and then quantifies captured bacteria by measuring bioluminescence induced by release of ATP from the bacterial cells. Results from this method are available within 1 h from the start of sample processing. Significant linear correlations were found between the IMS/ATP results and results from traditional culture-based methods for *E. coli* and enterococci enumeration for one location in California, two locations in North Carolina, and one location in Ohio (r values ranged from 0.87 to 0.97). No significant linear relation was found for a second location in California that treats a complex mixture of residential and industrial wastewater. With the exception of one location, IMS/ATP showed promise as a rapid method for the quantification of faecal-indicator organisms in wastewater.

Published by Elsevier Ltd.

1. Introduction

Current US Environmental Protection Agency (US EPA) approved methods for quantification of *Escherichia coli* (*E. coli*) and enterococci in recreational waters require 18–24 h to obtain results (US EPA, 2006b,c). This lag time can place the uninformed public at risk of exposure to high concentrations of bacteria when using recreational waters. The scientific community, as well as government regulators, have recognized the need to develop rapid methods for the detection of microbial indicators of faecal contamination (US Congress, 2007; US EPA, 2007). Several researchers have reported on alternatives to the traditional enumeration methods, including molecular methods, such as quantitative polymerase chain reaction (qPCR) and transcription mediated amplification (TMA) (Griffith et al., 2004; Noble and Weisberg, 2005). These techniques measure quantities of nucleic acids, which can come from both viable and nonviable cells, resulting in the potential for overestimation of the actual health risk as compared to traditional culture-based methods.

An alternative rapid method, developed by Lee and Deininger (2004) and modified by Bushon et al. (2009), uses antibody-coated magnetic beads and immunomagnetic separation (IMS) to concentrate target bacteria and then measures bioluminescence

* *Corresponding author*. Tel.: +1 614 430 7783; fax: +1 614 430 7777.
E-mail addresses: rnbushon@usgs.gov (R.N. Bushon), amgbrady@usgs.gov (A.M.G. Brady).
[1] Formerly at the US Geological Survey.

0043-1354/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.watres.2009.06.047

response to adenosine triphosphate (ATP) contained in the bacteria. In a review of rapid detection technologies by Noble and Weisberg (2005), the IMS/ATP method was highlighted as a technology that shows promise for accurately measuring faecal-indicator bacteria for recreational water-quality assessments. Because this method detects a chemical present in living cells, only viable *E. coli* or enterococci are included in the measurement, as ATP degrades rapidly upon cell death (Deininger and Lee, 2001). Advantages of this method include rapid results (1 h), simplicity of the equipment and assay (making technology transfer to agencies responsible for routine monitoring realistic), and the portability of the equipment (making field deployment a viable option). Additionally, the cost of equipment and supplies is considerably less than that of molecular methods (Noble and Weisberg, 2005). Although there are many advantages to the IMS/ATP method, there are also some limitations, including the type of antibody used. Polyclonal antibodies, which are derived from multiple cell lines, are commonly used and may react with other related microorganisms (Lee and Deininger, 2004). Additionally, the IMS/ATP method may be detecting viable organisms that contain ATP but are unable to be cultured on agar plates. This would ultimately affect the correlation between the IMS/ATP method and the traditional culture-based method results.

One common cause of beach closures in the United States is contamination of swimming areas by untreated or partially treated sewage from combined-sewer overflows, sanitary-sewer overflows, and/or discharges from malfunctioning sewage-treatment plants (US EPA, 2006a). The ability of a rapid method to accurately quantify levels of faecal contamination from wastewater is crucial for the protection of public health at swimming beaches and recreational rivers that are affected by wastewater contamination.

This paper describes the relationship between results from the IMS/ATP and traditional culture-based methods for *E. coli* and enterococci in 19 wastewater samples collected from March 2007 through June 2008 at five different geographical locations in the US states of Ohio, North Carolina, and California. Recent modifications to the IMS/ATP method, including changes to the *E. coli* antibody used, antibody-bead preparations, and reagents also are described.

2. Materials and methods

2.1. Study sites and wastewater sampling

Samples were collected from a total of five wastewater treatment plants (WWTP) in Ohio, North Carolina, and California. Table 1 lists information about design capacity, population served, and estimated percentages of input sources for each plant. So that temporal diversity of the wastewater could be accounted for, samples were collected intermittently over a period greater than 1 year, from March 2007 to June 2008. At each WWTP, a grab sample of primary-treated wastewater was collected into a sterile 1-l bottle. The samples were immediately placed on ice and shipped overnight to the US Geological Survey (USGS) Ohio Water Microbiology Laboratory (OWML) for analysis within 24 h of collection.

2.2. Traditional culture-based methods

Samples were analyzed by using the modified mTEC method (US EPA, 2006b) and the mEI method (US EPA, 2006c) for enumeration of *E. coli* and enterococci, respectively, to facilitate comparison with results from the IMS/ATP method. For *E. coli* enumeration, serial dilutions of the wastewater were made in phosphate buffered saline (PBS), filtered through 0.45-μm-pore-size filters (Advantec MFS, Inc, Dublin, CA), and placed on modified mTEC agar plates (Difco, Detroit, MI). After incubation for 2 h at 35 °C and 22 h at 44.5 °C, any colonies that were red or magenta were enumerated. For enterococci enumeration, dilutions of wastewater were filtered through 0.45-μm-pore-size filters and placed on mEI agar plates (Difco, Detroit, MI). After incubation for 24 h at 41 °C, any colonies with a blue halo were enumerated. The concentrations of *E. coli* and enterococci were reported in colony-forming units per 100 milliliters (CFU/100 ml).

2.3. Immunomagnetic separation/adenosine triphosphate method

The IMS/ATP method originally was described for *E. coli* in a report by Lee and Deininger (2004). Modifications to the original method, as well as a method for the detection of enterococci by IMS/ATP, are described in Bushon et al. (2009). For the current study, additional modifications were made, including the use of an IMS diluent (ImmTech, New Windsor, MD) which was designed to reduce nonspecific signal from samples with a complex matrix. The goat polyclonal anti-*E. coli* antibody described in Bushon et al. (2009) was no longer available; therefore, a rabbit polyclonal anti-*E. coli* antibody was used for the current study. Additionally, a commercial supplier was used to coat the beads with the appropriate antibody and supply the current study with antibody-coated beads in order to reduce variability in the coating process. Incorporation of these modifications into the IMS/ATP method is described below.

The same serial dilutions of wastewater samples that were made for the culture-based methods were used for the IMS/ATP method. A range of bacteria concentrations for each location was analyzed (Tables 2 and 3). To begin, 25 ml of the diluted sample were added to 15 ml of IMS diluent (ImmTech) in a 50-ml polypropylene centrifuge tube. For the IMS analysis, 100 μl of *E. coli* or enterococci antibody-coated beads (ImmTech) were added to the sample in the 50-ml tube. The tubes were rotated at 18 rpm for at least 30 min on a sample mixer (Invitrogen, Carlsbad, CA) to allow the target bacteria to attach to the antibody-coated beads. Tubes were then transferred to an MPC-50 magnetic particle concentrator (Invitrogen) and rotated by hand for 2 min in a fashion similar to that of method 1623 (US EPA, 2005) for *Cryptosporidium* and *Giardia*. The supernatant was decanted while the bacteria/bead complex adhered to the magnet. The sample was then washed with 5 ml of IMS diluent and rotated by hand on the magnet for another 2 min. After the supernatant was decanted, the bacteria/bead complex was resuspended in approximately 1.2 ml of PBS and transferred to 1.5-ml centrifuge tubes.

Please cite this article in press as: Rebecca N. Bushon et al., Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater, Water Research (2009), doi:10.1016/j.watres.2009.06.047

Table 1 – Wastewater treatment plant (WWTP) information.

Study sites (location)	Design capacity (million liters per day)	Population served	Residential/commercial input (percent)[a]	Industrial input (percent)[a]	Reference for input percentages
City of Delaware WWTP (Delaware, OH)	23	34000	95	5	Stanton (2009)
Morehead City WWTP (Morehead City, NC)	6.4	8700	98	2	Hamilton (2009)
Mason Farm WWTP (Chapel Hill, NC)	55	77000	98	2	Forney (2009)
Avalon WWTP (Avalon, CA)	4.5	3500	99	1	Matthews (2009)
Orange County Sanitation District (Fountain Valley, CA)	950	2500000	87	13	McGee (2009)

a Inputs are estimated percentages.

The 1.5-ml tubes were placed in an MPC-S magnetic particle concentrator (Invitrogen) for further concentration of the bacteria/bead complex. The remaining steps are described in detail in Bushon et al. (2009) with a brief description below. Somatic cell releasing agent (SRA) (New Horizons Diagnostics, Columbia, MD) was added to each tube to remove nonbacterial ATP. After a PBS wash, bacterial releasing agent (BRA) (New Horizons Diagnostics) was added to each tube to release the *E. coli* or enterococci ATP into solution. Luciferin-luciferase (LL, New Horizons Diagnostics), which reacts with ATP to produce light, was added to the concentrate containing the target bacteria ATP. A microluminometer (New Horizons Diagnostics) was used to measure luminescence in each sample, with results reported in relative light units per 100 milliliters (RLU/100 ml).

To test the quality of reagents and the potential for contamination through sample processing and to establish laboratory reporting limits, an equipment blank for each bacterium was run daily. The equipment blank consisted of PBS that was processed through the entire method.

2.4. *Statistical analyses*

Traditional culture-based method and IMS/ATP method results were $\log_{10}$ transformed prior to statistical analysis. Pearson's correlation coefficients were calculated to examine the linear relationship between the traditional method and the IMS/ATP method results. Statistical analyses were done in SAS (SAS Institute Inc., 1999) with an alpha level set at 0.05.

3. Results and discussion

Wastewater samples from the various locations were diluted and analyzed by using the IMS/ATP method and traditional culture-based methods for *E. coli* and enterococci enumeration. Luminescence, as determined by the IMS/ATP method (in RLU/100 ml), was plotted against concentrations, as determined by culture-based methods (in CFU/100 ml), for *E. coli* (Fig. 1) and enterococci (Fig. 2). Regression equations and coefficients of determination are listed in Fig. 1B–F for *E. coli* and Fig. 2B–F for enterococci. Pearson's correlation coefficients were determined for the relations between IMS/ATP and culture-based results for each of the locations and all locations combined as listed in Table 2 for *E. coli* and Table 3 for enterococci.

Dilutions of samples were prepared to encompass a wide range of concentrations to assess the relations between the methods at different levels of wastewater contamination. The numbers of samples collected at each location are listed in Tables 2 and 3 for *E. coli* and enterococci, respectively, along with the total number of IMS/ATP analyses. Replicates of most, but not all, dilutions were analyzed and treated as individual sample results in the correlation analysis.

Previous studies to evaluate the IMS/ATP method have shown strong, significant correlations between IMS/ATP results and those of the traditional culture-based method for faecal-indicator organisms. Lee and Deininger (2004) reported significant correlations at several freshwater beaches and a river for *E. coli* ($r = 0.96$). In a separate series of studies, investigators evaluated the IMS/ATP method with waters from a recreational river affected by discharges of stormwater, combined-sewer overflows, and incompletely disinfected wastewater from urban areas (Brady, 2007; Bushon et al., 2007, 2009). For these studies, Pearson's correlation coefficients indicated moderately strong, significant linear relations between results from the IMS/ATP and traditional culture-based methods for *E. coli* and enterococci (r values ranging from 0.59 to 0.77).

A total of 14 *E. coli* and 13 enterococci equipment blanks were processed during this study. The mean RLU for the *E. coli* blanks was 55, and the standard deviation was 21. For the enterococci blanks, the mean RLU was 112, and the standard deviation was 114. A laboratory reporting limit (LRL) was calculated for each bacterium by adding the standard deviation to the mean RLU. Sample RLUs below the LRL were not plotted in Figs. 1 and 2 and were not used in the correlation analysis because they were considered to be indistinguishable from equipment blanks. The number of analyses below the LRL and the range of concentrations in those analyses are listed in Tables 2 and 3.

Significant correlations and linear relations between IMS/ATP and culture-based results were found for all samples, except for the four samples from the Orange County Sanitation District (OCSD) in California. Relations between the IMS/ATP and traditional culture-based methods for *E. coli* and enterococci appear to be site specific, as demonstrated in Figs. 1A and

Please cite this article in press as: Rebecca N. Bushon et al., Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater, Water Research (2009), doi:10.1016/j.watres.2009.06.047

Table 2 – Sample data for each wastewater treatment plant (WWTP) including correlations between log_{10}-transformed culture-based concentrations (modified mTEC agar) and IMS/ATP results for *E. coli*, and the incidence and range of concentrations below the lower reporting limit (LRL).

WWTP location	Number of samples collected	Number of IMS/ATP analyses	Range of concentrations analyzed[a]	Pearson's correlation coefficient	P value	Number of analyses below LRL	Range of concentrations below LRL[a]
Delaware, OH	8	48	2.3–110000	0.89	<0.0001	5	4.3–30
Morehead City, NC	2	17	61–1300000	0.95	<0.0001	3	61–610
Chapel Hill, NC	1	10	220–2200000	0.96	<0.0001	0	n/a
Avalon, CA	2	19	18–2900000	0.91	<0.0001	4	29–290
Orange County, CA	4	29	14–160000	0.35	0.0648	0	n/a
All sites	17	123	2.3–2200000	0.69	<0.0001	12	4.3–610

n/a, not applicable.
a Concentrations reported in CFU/100 ml as determined by the culture-based method using modified mTEC agar.

2A. The polyclonal antibodies used in the study may react to a wide range of organisms, thus enabling different populations per location to be captured. The different sites likely encompass heterogeneous populations of organisms, thereby resulting in different regression equations. The OCSD results highlight the importance of evaluating the efficacy of the IMS/ATP method for the waters of interest. Doing so will ensure that the method will detect different levels of the contamination source in that particular water matrix.

The results for the OCSD wastewater samples are corroborated by two earlier studies conducted by Southern California Coastal Water Research Project (SCCWRP) in 2004 and 2007. The IMS/ATP method was one of four methods included in the 2004 method comparison study (Griffith et al., 2004). Wastewater from OCSD was used in the laboratory-created samples (environmental samples spiked with different amounts of OCSD wastewater). The IMS/ATP method consistently underestimated levels of enterococci in the test samples. Poor relations to the traditional culture-based method for enterococci could have resulted from one or a combination of the following: inhibitory compounds in the wastewater used to spike samples, incorrect calibration of the equipment used, or a poor-performing magnetic capture system that was designed specifically for the 2004 study (Griffith et al., 2004).

In the 2007 SCCWRP study, untreated wastewater from OCSD was also used to evaluate several rapid methods. The IMS/ATP method was also included in that study, using the modifications described in this paper; however, IMS/ATP results were not significantly correlated with those from culture-based methods in environmental samples spiked with different amounts of OCSD wastewater (data not shown). The OCSD serves the highest population of the locations tested in this study and treats the highest percentage of industrial waste. This complex wastewater source may contain inhibitory compounds that affect the relations between the two

Table 3 – Sample data for each wastewater treatment plant (WWTP) including correlations between log_{10}-transformed culture-based concentrations (mEI agar) and IMS/ATP results for enterococci, and the incidence and range of concentrations below the laboratory reporting limit (LRL).

WWTP location	Number of samples collected	Number of IMS/ATP analyses	Range of concentrations analyzed[a]	Pearson's correlation coefficient	P value	Number of analyses below LRL	Range of concentrations below LRL[a]
Delaware, OH	9	42	1.9–60000	0.87	<0.0001	14	1.9–46
Morehead City, NC	2	15	27–930000	0.96	<0.0001	5	27–270
Chapel Hill, NC	1	9	98–980000	0.97	<0.0001	1	98
Avalon, CA	2	18	2.7–270000	0.94	<0.0001	5	2.7–27
Orange County, CA	4	20	13–23000	−0.10	0.6665	4	170–1100
All sites	18	104	1.9–980000	0.69	<0.0001	29	1.9–1100

a Concentrations reported in CFU/100 ml as determined by the culture-based method using mEI agar.

Please cite this article in press as: Rebecca N. Bushon et al., Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater, Water Research (2009), doi:10.1016/j.watres.2009.06.047

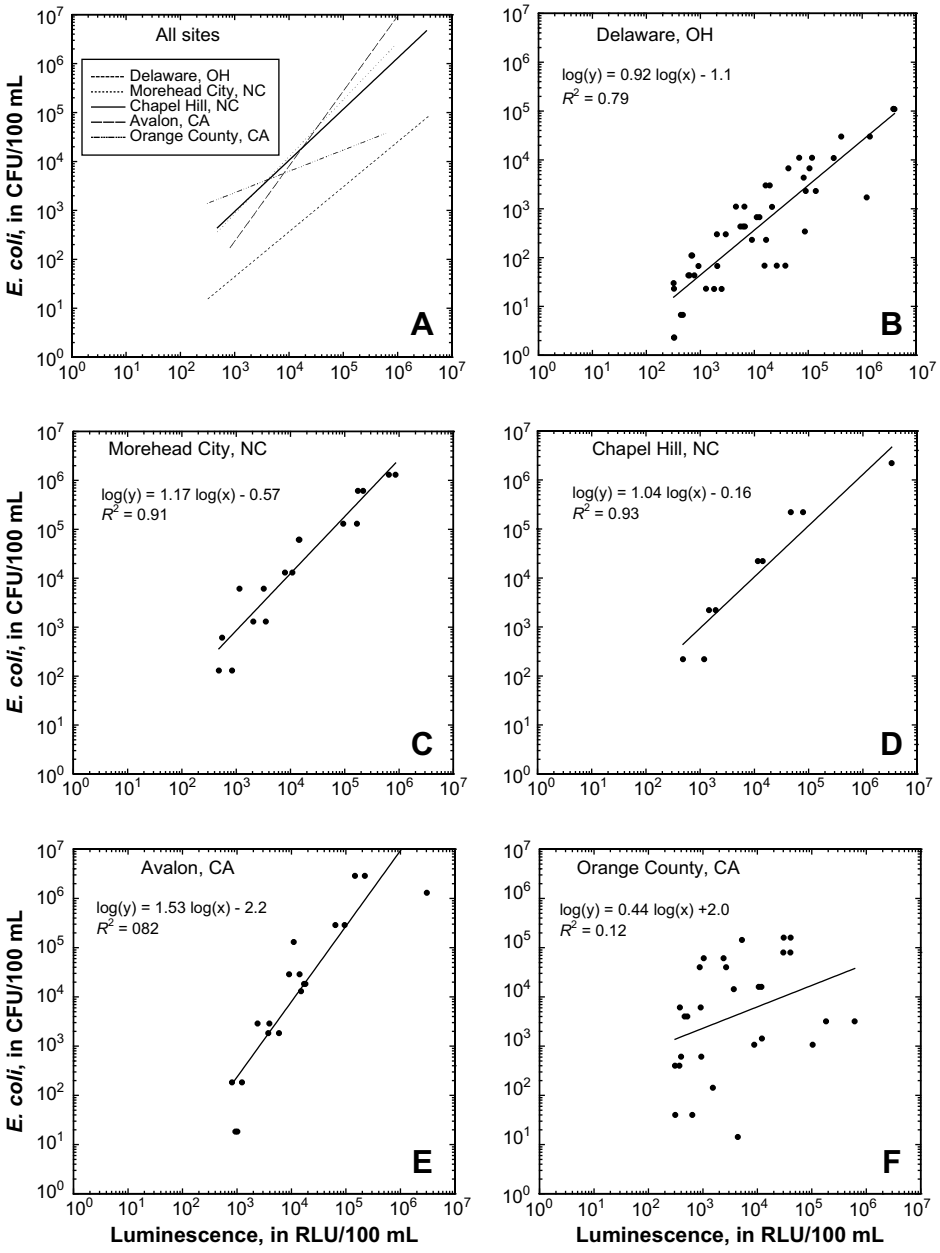

Fig. 1 – Regression lines and scatterplots of *E. coli* luminescence (in RLU/100 ml) measured by the IMS/ATP method versus *E. coli* concentrations (in CFU/100 ml) measured by the traditional culture-based method for wastewater samples from five locations plotted together (A) and each location separately (B–F).

methods possibly due to dissociation of antibodies from the beads, modification of the surface of the bacteria so that antibodies are unable to bind, or concentration of an inhibitor that affects the luciferin-luciferase reaction with ATP.

Sensitivity of rapid detection methods is an important factor in considering methods suitable for replacing traditional culture-based methods. As the current recreational water-quality standards for bathing waters range from 61 to 235 CFU/100 ml for *E. coli* and enterococci (US EPA, 1986), possible alternate methods must have detection limits below this level. In this study, a rigorous examination of the detection limit was not done; however, the lower detection limit was near 10 CFU/100 ml for many, but not all of the samples, for both *E. coli* and enterococci as seen in Figs. 1 and 2. In comparison, previous studies have reported lower detection limits for enterococci ranging from 5 to 27 CFU/sample for qPCR (Haugland et al., 2005; He and Jiang, 2005; Santo Domingo et al., 2003) and 10 CFU/100 ml for TMA (Morgan et al., 2007) in varying water matrices.

The IMS/ATP method shows promise as a feasible alternative method for the quantification of faecal-indicator bacteria in waters affected by wastewater contamination. Results of this study show strong, significant relations of IMS/ATP results to culture-based results in four of the five wastewater locations tested. Future studies are needed to evaluate IMS/ATP method performance in wastewater samples from

Please cite this article in press as: Rebecca N. Bushon et al., Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater, Water Research (2009), doi:10.1016/j.watres.2009.06.047

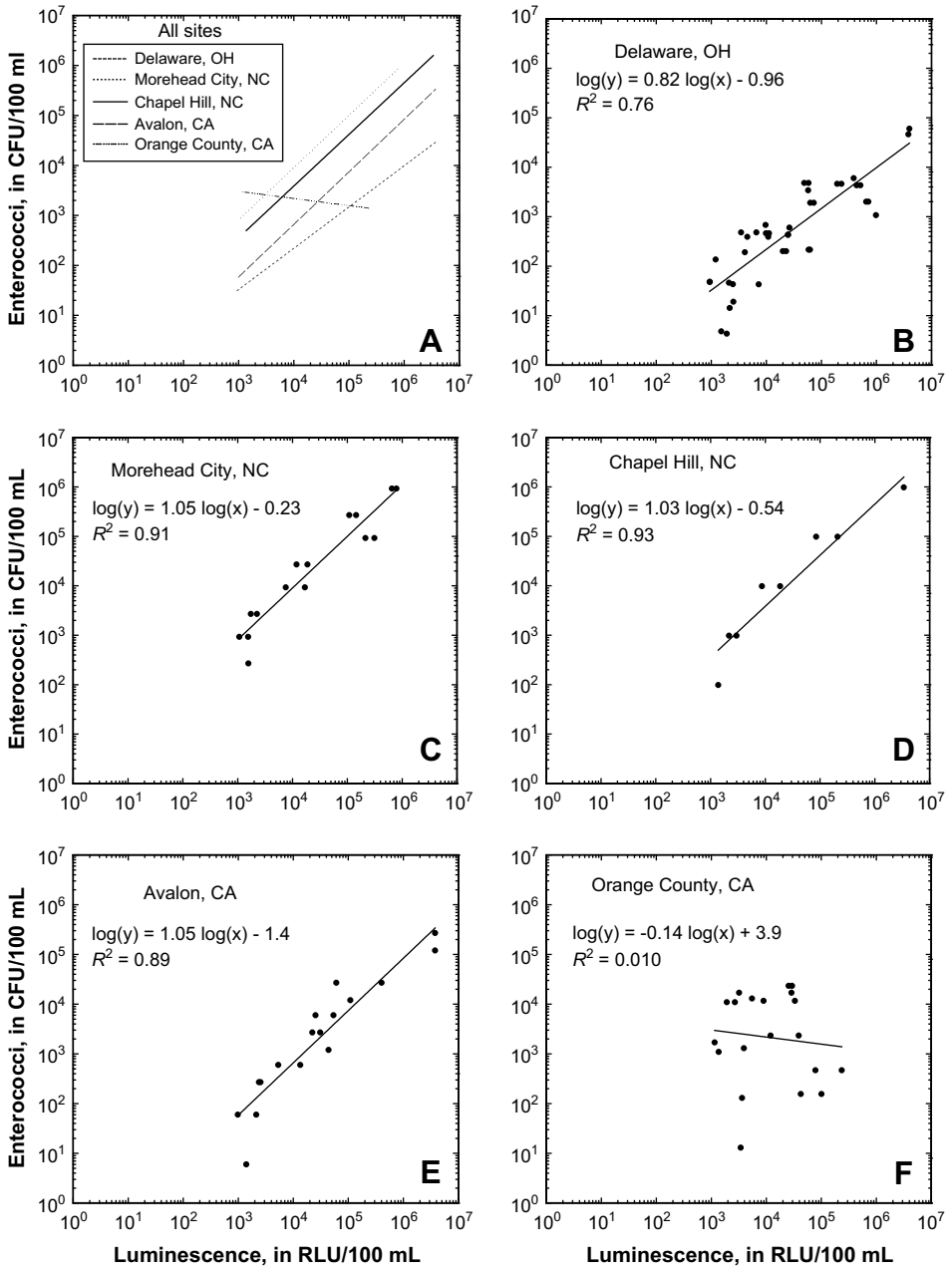

Fig. 2 – Regression lines and scatterplots of enterococci luminescence (in RLU/100 ml) measured by the IMS/ATP method versus enterococci concentrations (in CFU/100 ml) measured by the traditional culture-based method for wastewater samples from five locations plotted together (A) and each location separately (B–F).

additional sources, including those with complex inputs similar to OCSD. In the cases where IMS/ATP method results do not relate to culture-based methods in wastewater sources, additional studies should be done to document the relations of the methods in the environmental waters affected by these sources.

4. Conclusions

- Significant positive correlations between the IMS/ATP results and traditional culture-based methods for *E. coli* and enterococci enumeration in wastewater were found for four of the five locations tested.
- IMS/ATP is a promising new method for the rapid detection of bacteria in waters from recreational areas that are affected by wastewater contamination.
- The IMS/ATP method is not universally applicable and may be subject to inhibition from substances in complex wastewater sources.
- Sensitivity of the IMS/ATP method for *E. coli* and enterococci is comparable to molecular detection methods, with a lower detection limit near 10 CFU/100 ml for most, but not all of the samples.

Please cite this article in press as: Rebecca N. Bushon et al., Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater, Water Research (2009), doi:10.1016/j.watres.2009.06.047

Acknowledgements

The authors thank John Griffith and staff from the Southern California Coastal Water Research project for providing wastewater samples and guidance on method testing. We also thank Charles McGee from the Orange County Sanitation District; Angela Coulliette, formerly with University of North Carolina–Institute of Marine Sciences in Morehead City, NC; and David Love, formerly with University of North Carolina–Chapel Hill, for providing wastewater samples. This project was funded by the US Geological Survey National Water-Quality Assessment Program. The use of brand, firm, and trade names in this paper is for identification purposes only and does not constitute endorsement by the US Government.

REFERENCES

Brady, A.M.G., 2007. Rapid method for *Escherichia coli* in the Cuyahoga River. US Geological Survey Open-File Report 2007-1210, 5.

Bushon, R.N., Brady, A.M.G., Plona, M.B., 2007. Using a rapid method to predict recreational water quality at Cuyahoga Valley National Park, Ohio. Park Sci. 24, 89–93.

Bushon, R.N., Brady, A.M., Likirdopulos, C.A., Cireddu, J.V., 2009. Rapid detection of *Escherichia coli* and enterococci in recreational water using an immunomagnetic separation/adenosine triphosphate technique. J. Appl. Microbiol. 106, 432–441.

Deininger, R.A., Lee, J., 2001. Rapid determination of bacteria in drinking water using an ATP assay. Field Anal. Chem. Technol. 5, 185–189.

Forney, D., 2009. Personal communication. Mason Farm Wastewater Treatment Plant. 170 Old Mason Farm Road, Chapel Hill, NC 27517, USA.

Griffith, J.F., Weisberg, S.B., McGee, C.D., 2004. Evaluation of new, rapid methods for measuring microbiological water quality. Southern California Coastal Water Research Project Annual Report, 354–362.

Hamilton, S., 2009. Personal communication. Morehead City Wastewater Treatment Plant, 1000 Treatment Plant Road, Morehead City, NC 28557, USA.

Haugland, R.A., Siefring, S.C., Wymer, L.J., Brenner, K.P., Dufour, A.P., 2005. Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Res. 39, 559–568.

He, J.-W., Jiang, S., 2005. Quantification of enterococci and human adenoviruses in environmental samples by real-time PCR. Appl. Environ. Microbiol. 71, 2250–2255.

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

Matthews, S., 2009. Personal communication. Avalon Wastewater Treatment Plant, PO Box 1810, Pebbly Beach Road, Avalon, CA 90704, USA.

McGee, C., 2009. Personal communication. Orange County Sanitation District, PO Box 8127, Fountain Valley, CA 92728, USA.

Morgan, R., Morris, C., Livzey, K., Hogan, J., Buttigieg, N., Pollner, R., Kacian, D., Weeks, I., 2007. Rapid tests for detection and quantification of *Enterococcus* contamination in recreational waters. J. Environ. Monit. 9, 424–426.

Noble, R.T., Weisberg, S.B., 2005. A review of technologies for rapid detection of bacteria in recreational waters. J. Water Health 3, 381–392.

Santo Domingo, J.W., Siefring, S.C., Haugland, R.A., 2003. Real-time PCR method to detect *Enterococcus faecalis* in water. Biotechnol. Lett. 25, 261–265.

SAS Institute Inc., 1999. SAS OnlineDoc®, Version 8. SAS Institute Inc., Cary, NC.

Stanton, B., 2009. Personal communication. Delaware County Olentangy Environmental Control Center, 10333 Olentangy River Road, Powell, OH 43065, USA.

US Congress, 2007. Beach Protection Act of 2007. House of Representatives Bill 2537. Accessed November 29, 2007, at http://thomas.loc.gov/cgi-bin/query/z?c110:H.R.2537.

US Environmental Protection Agency, 1986. Ambient water quality criteria for bacteria–1986. EPA 440/5-84-002, Washington, DC.

US Environmental Protection Agency, 2005. Method 1623: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. EPA 815-R-05–002, Washington DC.

US Environmental Protection Agency, 2006a. Beach Pollution. Accessed November, 2008 at http://www.epa.gov/beaches/learn/pollution.html.

US Environmental Protection Agency, 2006b. Method 1603–*Escherichia coli* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar. EPA 821-R-06–011, Washington DC.

US Environmental Protection Agency, 2006c. Method 1600-Enterococci in water by membrane filtration using membrane-Enterococcus indoxyl-β-d-glucoside agar (mEI). EPA 821-R-06-009, Washington DC.

US Environmental Protection Agency, 2007. Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria. Office of Water, EPA 823-R-07–006, Washington DC.

Please cite this article in press as: Rebecca N. Bushon et al., Comparison of immunomagnetic separation/adenosine triphosphate rapid method to traditional culture-based method for *E. coli* and enterococci enumeration in wastewater, Water Research (2009), doi:10.1016/j.watres.2009.06.047