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Notes

A new, automated rapid fluorometric method for the detection of *Escherichia coli* in recreational waters

Andrew J. Bramburger [b,*], R. Stephen Brown [a], Jennifer Haley [b], Jeffrey J. Ridal [b]

[a] Department of Chemistry/School of Environmental Studies, Queens University, Kingston, ON K7L3N6, Canada
[b] St. Lawrence River Institute of Environmental Sciences, Cornwall, ON K6H4Z1, Canada

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ABSTRACT

Membrane filtration/culture techniques have been demonstrated to be reliable and broadly applicable for determination of fecal contamination in recreational waters. The time-consuming nature of culture techniques, however, is viewed as the major disadvantage of this type of analysis, and many authors have asserted the need for improved rapid-detection methods. In this study, we evaluated the performance of the ENDETEC™ TECTA™ B16, an automated fluorometry-based microbial detection system, by comparing its detection time and accuracy to those of two common culture-based methods, which are widely-used for recreational water quality monitoring in Canada. Our results demonstrated that *Escherichia coli* densities inferred by the TECTA™ method were generally in agreement with those generated by standard culture methods ($y = 1.19x + 0.002$, $R^2 = 0.89$) and under the current calibration regime, TECTA™ tended to slightly overestimate *E. coli* densities. In addition, TECTA™ was able to detect *E. coli* densities in exceedance of the Ontario Provincial Water Quality Objective for recreational waters in as little as 4 h (mean detection time = 7.03 h), representing a substantial improvement over traditional culture techniques. Our findings suggest that with improved calibration, TECTA™ may provide a viable, cost-effective, rapid alternative to culture approaches for the detection of fecal contamination in recreational waters.

Introduction

Fecal contamination of recreational waters has been implicated as an impairment of beneficial use at many locations within the Great Lakes/St. Lawrence basin (Dufour, 1984; Environment Canada and US EPA, 2006; Edge and Hill, 2007). Despite substantial improvement with respect to recreational water quality in many areas, monitoring and management of fecal contamination remain important facets of public health protection (Noble and Weisberg, 2005) and management agencies devote considerable resources to assessment of fecal contamination at beaches worldwide (Schiff et al., 2002; Colford et al., 2007). While fecal material may contain hundreds of bacterial taxa (McFarland, 2000; Fogarty et al., 2003, Dowd et al., 2008), coliforms, especially *Escherichia coli* (Migula, 1895), are widely regarded as the most reliable indicators of waterborne fecal contamination (Baudisova, 1997; Bartram and Rees, 2000; Kinzelman et al., 2003). Further, among bacterial indicators of recreational water quality, *E. coli* has been demonstrated to be the most reliable predictor of human health risk associated with swimming in fresh waters (Wade et al., 2003). As such, recreational water quality guidelines in Canadian jurisdictions on the Great Lakes continue to rely upon *E. coli* as an indicator of fecal contamination (Ontario Ministry of Health and Long-Term Care, 2008). Until recently, beach managers and public health agencies relied almost exclusively upon various membrane filtration and culture techniques despite the development of a wide variety of methods for the rapid determination of *E. coli* in environmental waters (Noble and Weisberg, 2005).

While various membrane filtration and culture techniques have been characterized as an adequate approach for inferring fecal contamination in recreational waters (Kabler and Clark, 1952; Ciebin et al., 1995; Grant, 1997), there are several issues that suggest the need to consider alternative methods. Culture techniques typically employed for *E. coli* determination at Canadian Great Lakes beaches include Differential Coliform Media (DCM) and Fecal Coliform media supplemented with 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (FC + BCIG, Ciebin et al., 1995). Both of these methods require that filter membranes be plated and incubated for 24 h. Often, 48 h can elapse between beach sampling and posting or removal of swimming advisories. Such lag times, coupled with the inherently binary nature of beach postings, present a substantial exposure risk for swimmers (Kim and Grant, 2004). Alternatively, erroneous postings can decrease beach visitation and may impact admissions revenue for beach managers and spending in surrounding communities.

In order to reduce or eliminate the consequences of time constraints associated with traditional culture techniques, a wide variety of alternative approaches have been developed, including the use of other fecal indicator taxa (Olapade et al, 2006; Newton et al., 2011), alternative culture substrata (Fricker et al., 1997), molecular rapid detection methods

* Corresponding author. Tel.: +1 218 720 2726.
E-mail address: abrambur@d.umn.edu (A.J. Bramburger).

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(Lavender and Kinzelman, 2009), and nowcast modeling (Nevers and Whitman, 2005; Francy et al., 2006; Francy, 2009). As with traditional culture techniques, these approaches also have their respective advantages and disadvantages. Although accelerated detection reagents such as Colilert® 18 can reduce incubation times by several hours (Chao et al., 2004), they can suffer from interference by non-target bacteria (Pisciotta et al., 2002). Several investigators have proposed the use of microbial taxa other than *E. coli* as indicators of fecal contamination (Fiksdal et al., 1985; Love and Sobsey, 2007). The relationship between these novel indicators and human health risk, however, remains largely uninvestigated (Colford et al., 2007), and several of these indicator taxa have been considered inappropriate for evaluation of non-human fecal contamination (Pisciotta et al., 2002). Noble and Weisberg (2005) asserted that the pool of potential non-bacterial microbial indicators at our disposal has been broadened by the recent development of technology-driven molecular and genetic techniques, including chemifluorometry and Quantitative Polymerase Chain Reaction (qPCR). Due to their ability to quantify non-cellular agents and viruses, and their lack of reliance on time-consuming culture incubations, these techniques represent the most promising avenues for rapid detection of fecal contamination. However, the majority of these advanced techniques require expensive equipment and specifically-trained, dedicated personnel (Noble and Weisberg, 2005). As such, these technologies often fall beyond the scope of most grassroots beach management and monitoring programs.

The ENDETEC™ TECTA™ B16, produced by Pathogen Detection Systems Inc. (Kingston, ON) is an automated microbial detection system that provides single-cell detection sensitivity for *E. coli* and total coliforms in water samples. The system consists of the TECTA™ B16 detection instrument and TECTA™ CCA test cartridges. The TECTA™ B16 is a self-contained bench top instrument capable of detecting the presence of *E. coli* or associated coliforms simultaneously in 16 individual incubation chambers. The TECTA™ CCA test cartridge is a self-contained sampling bottle and an analysis cartridge that contains proprietary pre-measured growth media and metabolic substrates specific to the target microbe (e.g. *E. coli*). A glucuronic acid conjugate is used as the substrate to detect the β-glucuronidase enzyme, the preferred indicator for *E. coli* in culture-based tests (Kilian & Buelow, 1976). The specificity for *E. coli* is therefore expected to match that of the DCM and FC + BCIG methods are used here for comparison. If the *E. coli* target is present, the enzyme acts on the substrate to release a hydrophobic, fluorescent metabolite. Background turbidity and matrix effects are mitigated by absorption of the fluorescent metabolite into an integrated polymer bead in the bottom of the cartridge where fluorescence is detected by the instrument. Higher concentrations of target microbes result in more rapid detection of fluorescent product. A negative result is returned if no fluorescence is detected within 18 h.

In this study, we investigated the ability of the TECTA™ B16 to estimate *E. coli* densities in recreational waters by comparing TECTA™ B16 fluorometrically-inferred *E. coli* densities to densities derived by standard membrane filtration techniques (DCM and FC + BCIG). We tested the hypothesis that there was no difference among *E. coli* densities inferred by three methods (TECTA™, DCM, FC + BCIG) for respective replicate subsamples of recreational waters from St. Lawrence River public swimming beaches. We also evaluated differences in detection time between the TECTA™ and culture methods in samples that were in exceedance of the Ontario Provincial Water Quality Objective (PWQO) of 100 *E. coli* CFU/100 mL. We anticipate that this technology, if proven, may represent a cost and labor-effective alternative for accurate, rapid determination of fecal contamination in recreational waters.

Methods

Instrument calibration

The TECTA™ B16 detection instrument was calibrated by the manufacturer using a series of Lake Ontario surface water samples taken from near Kingston, Ontario, with varying *E. coli* concentrations. *E. coli* density in water samples was determined using Idexx Colilert® 18 (Idexx Laboratories Inc. Westbrook, ME) as a reference test. The relationship between *E. coli* contamination level and time-to-detection (TTD) by the TECTA instrument was determined by linear regression ($y = 1.2856x + 11.322$, $R^2 = 0.70$).

Site description and sampling

A total of 43 water samples were taken from nine locations in the St. Lawrence River during August, 2012. Sampling locations included nearshore and swimming areas at four St. Lawrence River beaches located in the united counties of Stormont, Dundas and Glengarry, ON, Canada (Charlottenburg Park Beach, Crysler Beach, Farran Park Beach, and Glengarry Park Beach), as well as from a suspected *E. coli* point source within an inlet along the Cornwall, ON waterfront (Fig. 1). The point source was selected as a sample site in order to provide a location with a high bacterial load. Samples (1 L per site) were collected in accordance with the Ontario Beach Management Protocol (Ontario Ministry of Health and Long-Term Care, 2008) and placed on ice for transport to the laboratory. Upon returning to the lab, samples were homogenized and subsampled in triplicate for microbial analysis.

Microbial culture and enumeration

All samples were returned to the lab for *E. coli* analysis within 4 h of collection. Water samples from the point source location were serially diluted with a modified phosphate buffer solution (dilution range: 1:10 to $1:1.0 \times 10^{-4}$). Subsamples of 50 mL and 100 mL were obtained from the water samples for analysis by membrane filtration using DCM (Oxoid Ltd. Basingstoke, U.K.) and FC + BCIG (Oxoid Ltd. Basingstoke, U.K.) agar. DCM and FC + BCIG plates were incubated at 44.5 °C for 24 h, and *E. coli* colonies were subsequently counted under a dissecting microscope. Additional 100 mL subsamples were obtained for *E. coli* analysis by the TECTA™ at an incubation temperature of 44.5 °C. Time-to-detection of fluorescence by the TECTA™ instrument was compared to the standard calibration curve in order to infer *E. coli* density.

Statistical analysis

Inferred *E. coli* counts were log-transformed for all samples. We used linear regression analysis to determine the relationships between our standard enumeration method (DCM) and both an additional, widely-used culture method (FC + BCIG; Ciebin et al, 1995; Ontario Ministry of Health and Long-Term Care, 2008; Lyautey et al, 2010) and between the DCM and the TECTA™ method. In order to more comprehensively evaluate the influence of enumeration methods on inferred *E. coli* densities among methods, we used a Repeated Measures Analysis of Variance (rANOVA) with post-hoc Tukey's Honestly Significant Difference (HSD) tests comparing mean *E. coli* densities by method. Additionally, a two sample t-test was used to test for differences in detection times among enumeration methods for all cases in which the inferred *E. coli* density exceeded the PWQO of 100 CFU/100 mL in any of the subsamples. Statistical analyses were performed using JMP 7.0 for Windows (SAS Institute, 2007).

Results

A total of 43 water samples from 9 sites along the St. Lawrence River were analyzed for *E. coli* by three different detection methods. Inferred *E. coli* densities ranged from 0 to 3.4×10^6 CFU/100 mL for the DCM method, 1 to 4.1×10^6 CFU/100 mL for the FC + BCIG method, and 1 to 1.4×10^7 CFU/100 mL for the TECTA™ method. The lowest *E. coli* densities tended to occur at Glengarry Park and Charlottenburg Park Beaches while the highest counts occurred consistently in samples from the Cornwall waterfront point source.

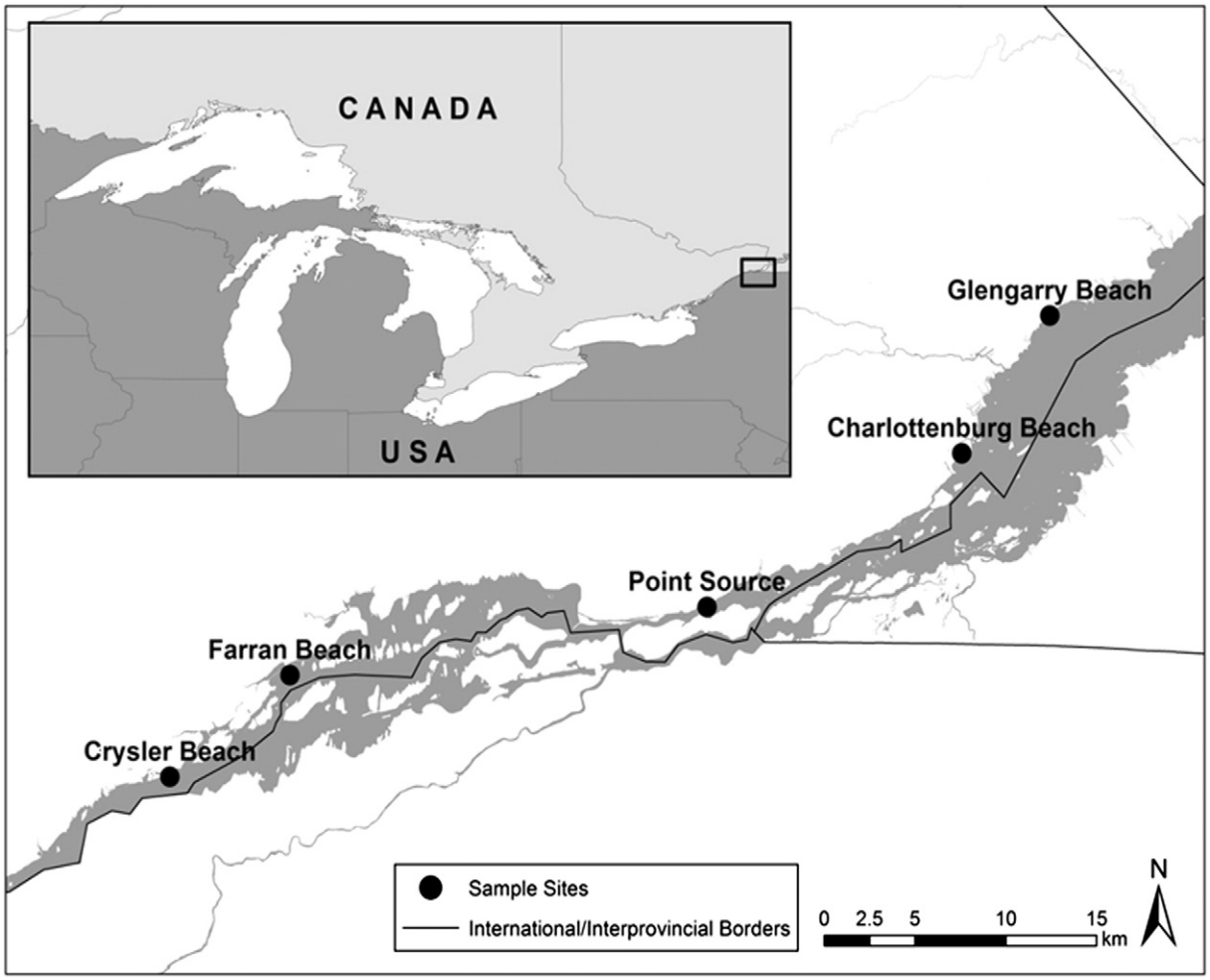

Fig. 1. Map of the international section of the St. Lawrence River showing the locations of sample sites including Crysler Beach, Farran Park Beach, Charlottenburg Park Beach, Glengarry Park Beach, and a known point source of *E. coli* at an inlet in along the Cornwall waterfront.

Linear regression analysis demonstrated strong, positive relationships between the standard DCM method and both the other membrane filtration methods (FC + BCIG; $y = 1.02x - 0.03$, $R^2 = 0.96$, $p < 0.0001$, Fig. 2a) and the fluorescence method (TECTA; $y = 1.19x + 0.02$, $R^2 = 0.89$, $p < 0.0001$, Fig. 2b). A rANOVA revealed that there were significant differences in mean inferred log *E. coli* density among samples ($n = 43$, Exact $F = 76.6$, $p < 0.0001$) and among enumeration methods ($n = 43$, Exact $F = 9.4$, $p = 0.0002$). Post-hoc Tukey's HSD tests showed that the TECTA™ method generated significantly higher estimates of log *E. coli* densities than either the DCM or BCIG methods ($z = 0.50$, $p < 0.05$; $d = 0.46$, $p < 0.05$), respectively (Fig. 3).

The mean detection time for samples in exceedance of the Ontario PWQO by the TECTA™ method was 7.03 h ($\sigma = 2.64$ h) with a maximum detection time of 9.38 h. A one-sample t-test comparing detection times of the TECTA™ method with those of the membrane filtration techniques in cases where any analysis detected an exceedance of the PWQO showed that TECTA™ detection times were significantly shorter than those for the membrane filtration methods ($n = 15$, $t = -24.9$, $p < 0.0001$).

Discussion

In this study, we evaluated the performance of the ENDETEC™ TECTA™ B16 automated microbial detection system by comparing its detection accuracy, precision, and turnaround time to those of two standard membrane filtration techniques (DCM and FC + BCIG). Linear regression analysis demonstrated strong agreement between the FC + BCIG and DCM-inferred *E. coli* density ($y = 1.02x$, $R^2 = 0.96$, $p < 0.0001$) and suggested that either of these methods may be considered as appropriate controls against which to test other detection methods. Although TECTA™ inferred *E. coli* density was also positively related with DCM-inferred *E. coli* density ($y = 1.19x$, $R^2 = 0.89$, $p < 0.0001$), the relationship exhibited slightly more variability and the TECTA™ method tended to over-estimate *E. coli* density, especially at the high end of the DCM-inferred density range. The strength of the relationship, however, suggests that the agreement between estimates generated by these methods may be improved by re-calibrating the TECTA™ method with local surface water as opposed to current calibration curves derived from Lake Ontario waters. An examination of residuals from this regression analysis revealed only small deviations from the regression line within the region near 100 CFU/mL. This finding suggests that the TECTA™ method is capable of providing high-precision estimates of *E. coli* density within the range of greatest interest to Ontario beach management agencies. Assuming DCM-inferred densities are representative of actual conditions, the TECTA™ method exhibited an overall accuracy of 83.3%, a sensitivity of 88.9%, and a specificity of 80.0%, with respect to properly-classified exceedances of the Ontario Provincial Water Quality Objective. The FC + BCIG method was more effective with an overall accuracy of 95.8%, a sensitivity of 100% and a specificity of 93.3%. We note that the accuracy and specificity of the TECTA™ method were negatively affected by its tendency to overestimate *E. coli* densities, and it is anticipated that the comparatively low accuracy of this method will likely be improved with re-calibration. Further, the effectiveness of re-calibration could be improved through the use of DCM

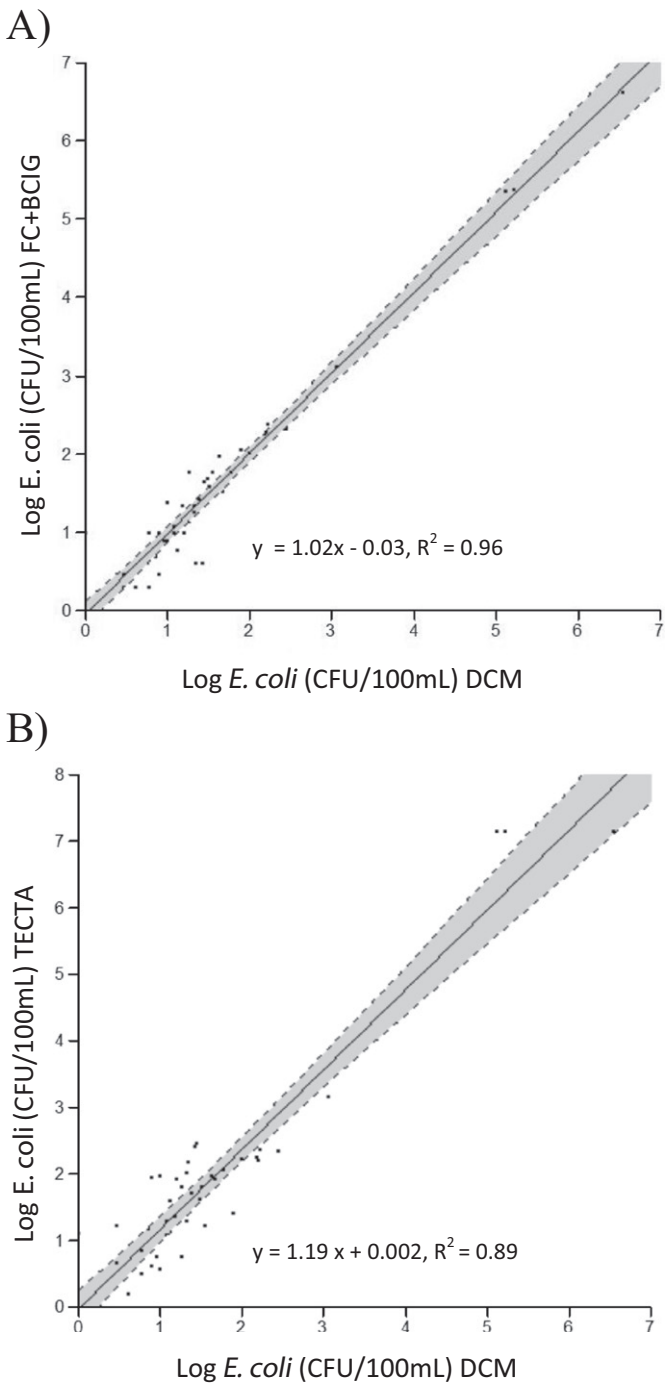

Fig. 2. A) Linear regression plot of log *E. coli* density detected by the FC + BCIG standard culture method vs. log *E. coli* density detected by the DCM standard culture method. Regression results suggest strong agreement between these two culture techniques. B) Linear regression plot of log *E. coli* density detected by the TECTA™ fluorometry method vs. log *E. coli* density detected by the DCM standard culture method. Shaded areas represent the 95% CI for the linear relationships between variables.

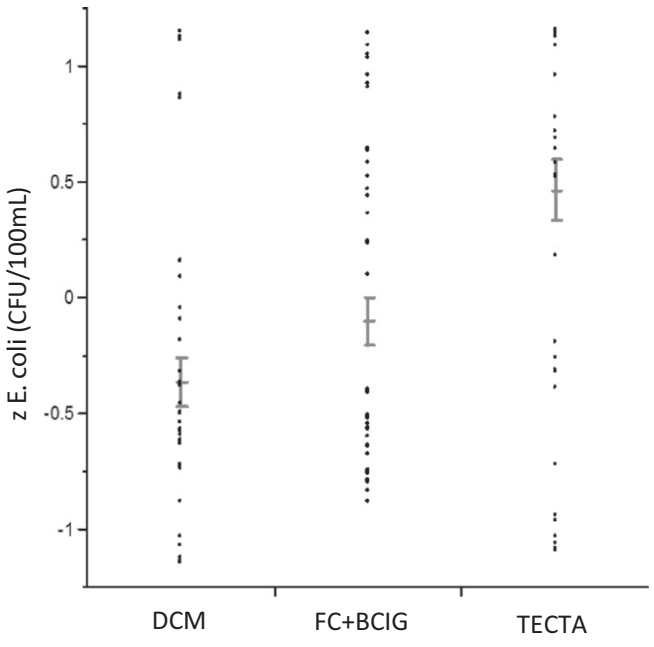

Fig. 3. Differences in mean *E. coli* density as inferred by DCM, FC + BCIG, and TECTA™ enumeration methods. Results shown are normalized to sample mean *E. coli* densities. Points represent individual subsample measurements, error bars represent the 95% CI of the mean. Repeated measures ANOVA revealed that TECTA™ *E. coli* estimates are significantly higher than estimates from both standard membrane filtration techniques.

or a similar culture method in the place of the Colilert® 18 method in generating a calibration curve.

Repeated Measures ANOVA results concurred broadly with linear regression results. Differences observed among sample sites were anticipated, and were reflective of trends observed in beach monitoring datasets from the 2012 swimming season (EOHU unpublished data, SLRIES unpublished data). Differences among enumeration methods were consistent with linear regression results, with TECTA-inferred *E. coli* densities significantly higher than corresponding results from either membrane filtration method. Over-estimation of bacterial densities by the TECTA™ method occurred consistently across all samples, and was most pronounced in samples with high *E. coli* densities.

The primary drawback of traditional membrane filtration and culture techniques for the detection of *E. coli* is the time consuming nature of plate incubations and colony counting. Our results demonstrated that in cases where the PWQO was exceeded, TECTA™ results were available more than 10 h sooner, on average, than results from culture analysis. These time savings in the laboratory can translate to about 24 h of time savings for management decisions, thereby reducing public health risk and/or protecting beach admission revenues. For example, using common culture techniques, samples collected on a Monday afternoon could be filtered and plated by Monday evening, then cultured for 24 h and read, at earliest, on Tuesday evening. Results would be available for management actions to be implemented on Wednesday morning. Using the TECTA™ method, any exceedances that occurred in Monday afternoon samples would be detected overnight, and results would be available for management actions to be implemented on Tuesday morning.

The current recreational water quality literature uniformly identifies the need for improved rapid detection capability for fecal contamination (Freier and Hartman, 1987; Schiff et al., 2002; Noble and Weisberg, 2005; McQuaig et al., 2006; Colford et al., 2007), but it also illustrates the drawbacks associated with the cost and labor-intensiveness of many recently-developed methods (Noble and Weisberg, 2005). In this study, we have demonstrated that the fluorometry method employed by the ENDETEC™ TECTA™ B16 automated microbial detection system represents an accurate, precise, and relatively rapid method for the enumeration of *E. coli* in surface waters. We anticipate that with further adjustment of the *E. coli* density/detection time curve, this method will be comparable in effectiveness to other membrane filtration/culture methods including FC + BCIG. The relatively low cost of the instrument itself, the use of inexpensive consumables, and the ease-of operation make this a promising technique that could become a viable alternative to traditional techniques for recreational water quality monitoring with further testing.

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