Comparison of bacterial indicator analysis methods in stormwater-affected coastal waters

Rachel T. Noble[1], Molly K. Leecaster[2], Charles D. McGee[3], Stephen B. Weisberg, and Kerry Ritter

ABSTRACT - Membrane filtration (MF) and multiple tube fermentation (MTF) have been used for decades to measure indicator bacteria levels in beach water samples, but new methods based on chromogenic substrate (CS) technology are becoming increasingly popular. Only a few studies have compared results among these methods, and they have generally been based on samples collected from a limited number of sites during dry weather. In this study, samples were collected from 108 sites the day after a major rainstorm and three indicator bacteria (total coliforms, fecal coliforms or *E. coli*, and enterococci) were each measured using MF, MTF, and CS. Sampling sites were selected using a stratified random design, stratified by open sandy beach, rocky shoreline, and beach areas near urban runoff outlets. The CS was found to be highly correlated with both MF and MTF for all three indicators regardless of whether the samples were taken along open shoreline or near a runoff outlet. While correlated, total coliform values were slightly higher using CS, consistent with other studies that have demonstrated false positives with this method. Fecal coliform values were 12% lower with CS, reflecting specificity of the CS method for *E. coli* rather than the entire fecal coliform group. No significant differences were observed for enterococci, although some differences were observed within specific laboratories. Differences for all of these indicators were small enough that, when assessed categorically, there was more than 90% agreement between CS and either MF or MTF methods as to whether State of California Beach Water Quality Standards were met.

[1]*UNC-Chapel Hill Institute of Marine Sciences, 3431 Arendell St., Morehead City, NC 28557*

[3]*Idaho National Environment and Engineering Laboratory, 2525 N. Fremont Ave. Idaho Falls, ID 83415*

[3]*Orange County Sanitation District, 10844 Ellis Ave., Fountain Valley, CA 92708*

INTRODUCTION

The coastal ocean is an important economic and recreational resource that is influenced by human activities. Treated wastewater discharges, industrial inputs, and non-point source surface runoff all affect coastal water quality and create the impetus for extensive water quality monitoring programs (Schiff *et al.* 2002). The main criterion for assessing the potential health risk of recreational waters to swimmers is the density of indicator bacteria. Although indicator bacteria do not necessarily cause illness, they are abundant in human waste where pathogenic organisms, such as pathogenic bacteria, viruses, and parasites are also likely to exist. The bacterial groups most frequently used as indicators of fecal contamination are total coliforms (TC), fecal coliforms (FC, of which *E. coli* are a subset), and enterococci (EC).

Indicator bacteria have historically been measured using either membrane filtration (MF) or multiple tube fermentation (MTF), although chromogenic substrate (CS) methods, such as those manufactured by IDEXX Laboratories, Inc., have recently been gaining popularity. The allure of the CS test is that it is more rapid, providing results in as little as 18 h compared to the 24-96 h required for traditional methods. It has the additional advantage of being less expensive.

Several studies have compared results among these methods (Abbott *et al.* 1998, Budnick *et al.* 1996, Eckner 1998, Palmer *et al.* 1993, Bej *et al.* 1991, Covert *et al.* 1989, and Noble *et al.* 2003) and have generally reported high comparability. These studies, though, have mostly been conducted under dry-weather conditions. Land-based runoff that results from rainfall has the potential to interfere with these tests because it contains high levels of suspended solids, a problem particularly when using MF.

Furthermore, stormwater runoff and marine waters harbor native bacteria, such as *Aeromonas*, *Vibrios Pseudomonas*, and *Flavobacterium*, which can produce MUG+ reactions and potentially lead to false positives in the CS test (Pisciotta *et al.* 2002, Landre *et al.* 1998, Petzel and Hartmann 1986, Hidalgo *et al.* 1977).

Rainfall affects ocean water quality more dramatically in southern California than in other parts of the country because the coast is highly urbanized and the flood control system is independent of the sewage system, carrying urban runoff directly to the beach after a storm (Noble *et al.* 2003). In this study, samples were collected throughout southern California immediately after a rain event to compare results from IDEXX kits, which are a commercial application of the CS method, to the more routinely used MF and MTF methods. Comparisons were conducted for EC, FC, and TC.

METHODS

Samples were collected from 108 sites along the southern California coastline on February 22, 2000, which was one day after a storm that produced between 2.7 and 7.6 cm of rain over the entire region. This rain event was sufficient to induce flow in all major runoff outlets to the ocean. The sample sites were selected using a stratified random sampling design, stratified by open sandy beach (35 sites), rocky shoreline (15 sites), and beach areas within 100 meters of a freshwater outlet (58 sites). All samples were collected in ankle-deep water on an incoming wave, with the sampler positioned downstream from the bottle and the mouth of the bottle facing into the current.

Samples were split and processed using the standard operating procedures of each of the six laboratories that participated in the study, and the IDEXX CS kits, Colilert® and Enterolert®. The methods used included 9221B, C, and E; 9222B and D; and 9230B and C in Standard methods for the examination of water and wastewater, APHA, AWWA, WEF, 18th edition, 1995, and EPA Method 1600 for EC (APHA 1995). Not all laboratories used all methods on all samples, yielding 95 pairwise comparisons for TC, 80 for FC (the IDEXX method is specific to *E. coli*), and 61 for EC.

Three approaches were used to compare bacterial concentration data from CS methods with those from MF and MTF methods. The first was Pearson correlation after log transformation. The second was Thiel's (1958) sequential test for consistency and agreement, which is based on the following regression:

$$\log(STD) - \log(CS) = b_0 + b_1 \log(CS) + error$$

The method first assesses consistency, or whether differences between two methods are concentration independent, by testing whether b_1 differs from zero. Agreement, or whether the methods report similar bacterial concentrations for a given sample, is assessed by testing whether $b_0 = 0$. Significance levels were Bonferroni-adjusted for multiple comparisons within each indicator. Significant differences were reported as the median ratio of the standard method (STD) to CS, which defines a multiplicative adjustment necessary to produce similar bacterial concentrations between methods. Distribution-free simultaneous confidence intervals for this ratio were calculated using SAS's PROC UNIVARIATE. Since several laboratories were included in the study, we tested for the presence of laboratory effects prior to applying the Thiel's test. A laboratory effect was observed in only comparison, for which we performed Theil's test separately by laboratory.

The third analysis was a categorical comparison, assessing the consistency of sample classification with respect to the State of California's single sample recreational water quality standards (10,000 MPN or cfu/100 mL for TC, 400 MPN or cfu/100 mL for FC, and 104 MPN or cfu/100 mL for EC).

RESULTS

The CS results were highly correlated with both MF and MTF results for all three indicators, exceeding 0.91 except for the comparison of FC by MTF (Table 1, Figure 1a-c). Correlations between methods were high regardless of whether the samples were collected on open beaches or near freshwater outlets, although the correlation was somewhat lower for FC at freshwater outlets.

The consistency analysis yielded non-significant slope coefficients across all indicators and methods, indicating that any differences between methods were concentration independent. The agreement analysis, which assesses whether the methods provide similar results, varied by method and indicator

Table 1. Correlation between IDEXX and other routinely used methods to assess marine recreational water quality. Results are given overall, segregated by method, and segregated by shoreline type.

	Fecal Coliforms	Total Coliforms	Enterococci
Overall	0.91	0.91	0.92
By Method			
Membrane Filtration	0.92	0.92	0.93
Multiple Tube Fermentation	0.79	0.91	NA
EPA 1600	NA	NA	0.94
By Site Type			
Open Beaches	0.95	0.92	0.92
Near Runoff Outlets	0.84	0.92	0.93

species (Table 2). For TC, both MF and MTF yielded bacterial concentrations close to one-half of those reported by CS. The median ratio between CS and the other methods was 0.63 for MF and 0.55 for MTF, although only the MTF results were significantly different from CS.

For FC, MF results were statistically indistinguishable from CS but MTF concentrations were significantly larger than those of CS. This difference results because the IDEXX CS method is specific to *E. coli*, which is a subset of the FC group.

For EC, we observed a marginally significant laboratory effect and therefore conducted the analysis separately by laboratory. We found that one laboratory tended to report larger EC concentrations using the standard method, while the other tended to report larger concentrations using CS, but within-laboratory variability was large in both cases. Only for the laboratory reporting that CS produced higher values was the difference statistically significant, but the difference was only marginally significant. When the analysis was repeated using data from both laboratories combined, there was no significant difference between methods.

When examined categorically, we found greater than 90% agreement between methods with respect to the State of California's beach single-sample recreational water quality standards for all three indicators (Table 3). The greatest agreement occurred for TC, with 95% agreement; the 5% of samples that disagreed exceeded the standard using CS but did not exceed the State water quality standard using either MF or MTF. For FC, 8% of the samples disagreed, with most having higher results using MF or MTF. The greatest disagreement (10%) was found for EC, although there was no consistent pattern to these disagreements with an even split between CS and MF or MTF being higher.

DISCUSSION

Our general finding that the IDEXX CS method yielded comparable results to MF and MTF is consistent with that of several previous researchers (Abbott *et al.* 1998, Budnick *et al.* 1996, Eckner 1998, Palmer *et al.* 1993, Bej *et al.* 1991, Covert *et al.* 1989, Noble *et al.* 2003). Our study extends these previous efforts by sampling a variety of location types during a period of high land-based runoff, allowing us to sample a range of bacterial concentrations from near detection limits to more than a thousand times State of California single-sample standards. The

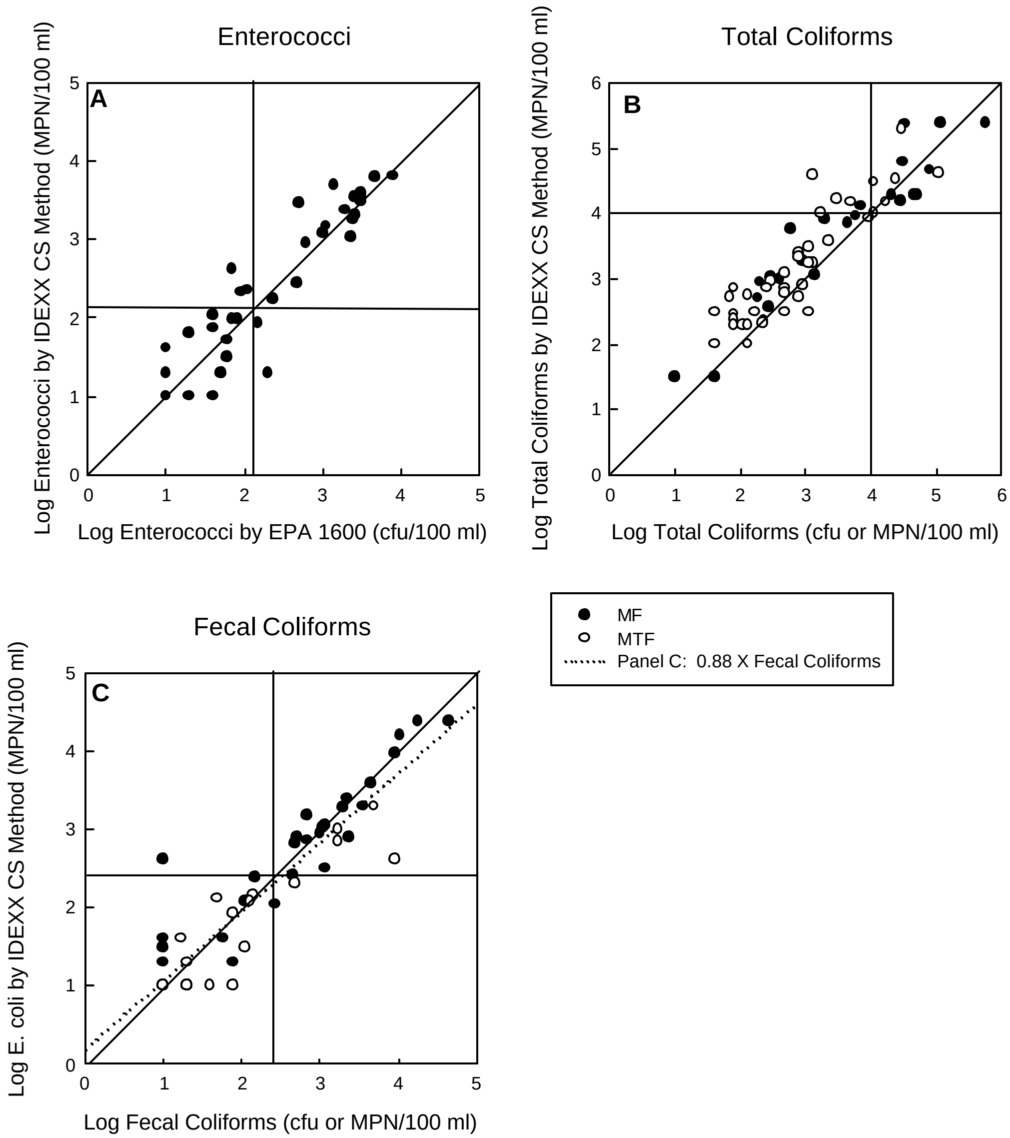

Figure 1. Membrane filtration and multiple tube fermentation results versus IDEXX results for (a) enterococci, (b) total coliforms, and (c) fecal coliforms. Diagonal lines represent one-to-one relationship. Horizontal and vertical lines are State of California standards.

comparability of our results over these large ranges, particularly at a time when interferences are likely to be greatest, provides additional assurance that the CS method compares well with MF and MTF under a wide array of sampling conditions.

Although we found a good agreement between results produced by CS methods versus those by MF or MTF methods, there were some differences. For instance, TC results using CS were approximately 1.6 times higher than for both MF and MTF methods (Figure 1b), consistent with results reported by Palmer *et al.* (1993) and Pisciotta *et al.* (2002). It has been suggested that the IDEXX method yields false positives with members of *Vibrio* and *Aeromonas* (Davies *et al.* 1995, Landre *et al.* 1998, and Pisciotta *et al.* 2002), which could explain the higher values.

To assess whether this difference resulted from inaccurate identification by CS, the wells from four CS samples were subjected to species verification using the Vitek System (bioMerieux Vitek, Inc., Hazelwood, MO). The Vitek System is a self-contained, fully automated *in-vitro* testing system for microbial identification. Test cards contain selected dried media in 30 tiny wells molded into the plate, where growth and biochemical reactions cause

Table 2. Median ratio and associated confidence interval of the ratio between results from membrane filtration (MF) or multiple-tube fermentation (MTF) and chromogenic substrate kits (CS).

Total Coliforms:			
Ratio	Median	Lower Limit	Upper Limit
MF/CS	0.62	0.40	1.29
MTF/CS	0.55	0.36	0.79
Fecal Coliforms:			
Ratio	Median	Lower Limit	Upper Limit
MF/CS	1.00	0.76	1.18
MTF/CS	2.00	1.00	2.51
Enterococci:			
Ratio	Median	Lower Limit	Upper Limit
MF (EPA 1600)/CS (Laboratory 1)*	1.75	0.50	2.50
MF (EPA 1600)/CS (Laboratory 2)*	0.67	0.11	1.00

*Laboratory effects significant.

Table 3. Threshold agreement between methods. Numbers represent the percent of samples within each category.

	FECAL COLIFORMS	
	MF or MTF< 400	MF or MTF> 400
IDEXX<400	55	6
IDEXX>400	2	37
	TOTAL COLIFORMS	
	MF or MTF<10,000	MF or MTF>10,000
IDEXX<10,000	64	0
IDEXX>10,000	5	31
	ENTEROCOCCI	
	MF or MTF<104	MF or MTF>104
IDEXX<104	38	4
IDEXX>104	6	52

MF - Membrane filtration.
MTF - Multiple tube fermentation.

changes to occur in color and turbidity in the wells. Two of the samples subjected to Vitek analysis were ones in which the CS method indicated that State of California standards were exceeded while the EPA 1600 method did not, and vice versa for the other two samples. In all four cases, the CS result was confirmed. These results are in contrast to Pisciotta *et al.* (2002), who found extensive false positives when conducting species confirmation of CS results. Pisciotta *et al.* (2002) conducted their work in south Florida and suggested that their findings might be specific to warm subtropical waters. The similarity of our findings to those of Palmer *et al.* (1993), who conducted their work in the colder southern California waters, would seem to support this suggestion. Further verification analyses will be useful in assessing whether these differences are truly geographically mediated.

A more systematic difference in our methods comparison was the lower fecal coliform values recorded using CS, which is a reflection of the specificity of the IDEXX CS method to measurement of *E. coli*, rather than all fecal coliforms. Regression analysis between CS results and MF/MTF results suggests that *E. coli* constituted 88% of fecal coliforms in this study (Figure 1C). The State of California Department of Health Services has suggested that because of the difference in target bacteria between methods, measurements of *E. coli* using IDEXX should be increased by 20% when comparing results to a fecal coliform standard. The results of this study suggest that this is conservative on average, but we also observed considerable variability in the percentage of FC that were *E. coli*. Some of this is probably due to measurement variability, but the relationship between the two could also be sample-type specific and suggests the need for additional study of this conversion factor.

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