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

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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 (TCs), fecal coliforms (FCs) or *E. coli*, and enterococci (EC)) 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 results were 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, TC values were higher using the CS method, consistent with other studies that have demonstrated false positives with this method. FC values were 12% lower with CS, reflecting the specificity of the CS method for *E. coli* rather than for the entire FC group. No significant differences were observed for EC, 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 methods and either MF or MTF methods as to whether State of California Beach Water Quality Standards were met or exceeded.

Keywords: Indicator; Bacteria; Coliforms; Enterococci; Membrane filtration; *E. coli*

1. 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 [1]. 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 (TCs), 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

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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 [2–9] 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 *Flavobacteria* spp., which can produce MUG+ reactions and potentially lead to false positives in the CS test [10–13].

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 [7,8]. 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.

2. Methods

Samples were collected from 108 sites along the southern California coastline on 22 February 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 m 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®, Colilert-18®, and Enterolert®. The MF and MTF 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 [14]. Not all laboratories used all methods on all samples, yielding 95 pairwise comparisons for TC, 80 for FC (where IDEXX is treated locally as a substitute for an FC method, even thought it is specific to *E. coli*), and 61 for EC. Salinity was measured for a subset of samples, using a Vista Refractometer (Model#A366ATC).

Three approaches were used to compare bacterial concentration data from CS methods with those from MF and MTF methods. The first was Pearson correlation (r) after log transformation. The second was Thiel's [15] sequential test for consistency and agreement, which is based on the following regression:

$$\log(\mathrm{STD}) - \log(\mathrm{CS}) = \beta_0 + \beta_1 \log(\mathrm{CS}) + \mathrm{error}.$$

The method first assesses consistency, or whether differences between two methods are concentration independent, by testing whether β_1 differs from zero. Agreement, or whether the methods report similar bacterial concentrations for a given sample, is assessed by testing whether $\beta_0 = 0$. Significance levels ($\alpha = 0.05$) were Bonferroni adjusted for multiple comparisons within each indicator. Significant differences were reported as the median ratio of either the MF or MTF method to CS, which defines a multiplicative adjustment necessary to produce similar bacterial concentrations between methods (for a more detailed description of Thiel's method see [16]. 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 one 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). Contingency tables of the percent of classifications above and below the water quality standard based on CS and either MF or MTF were computed for each of the three indicators.

3. Results

The CS results were highly correlated with both the MF and MTF results for all three indicators, with r-values exceeding 0.91 except for the comparison of FC by MTF (Table 1, Fig. 1). 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. Salinity did not appear to

Table 1
Pearson correlation coefficient (r) between chromogenic substrate kits and other routinely used methods to assess marine recreational water quality[a]

	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

[a] Results are given overall, segregated by method, and segregated by shoreline type.

have any consistent effect on the performance of the methods.

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 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 0.62 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 CS method from IDEXX Laboratories, Inc., is specific to *E. coli*, which is a subset of the FC group.

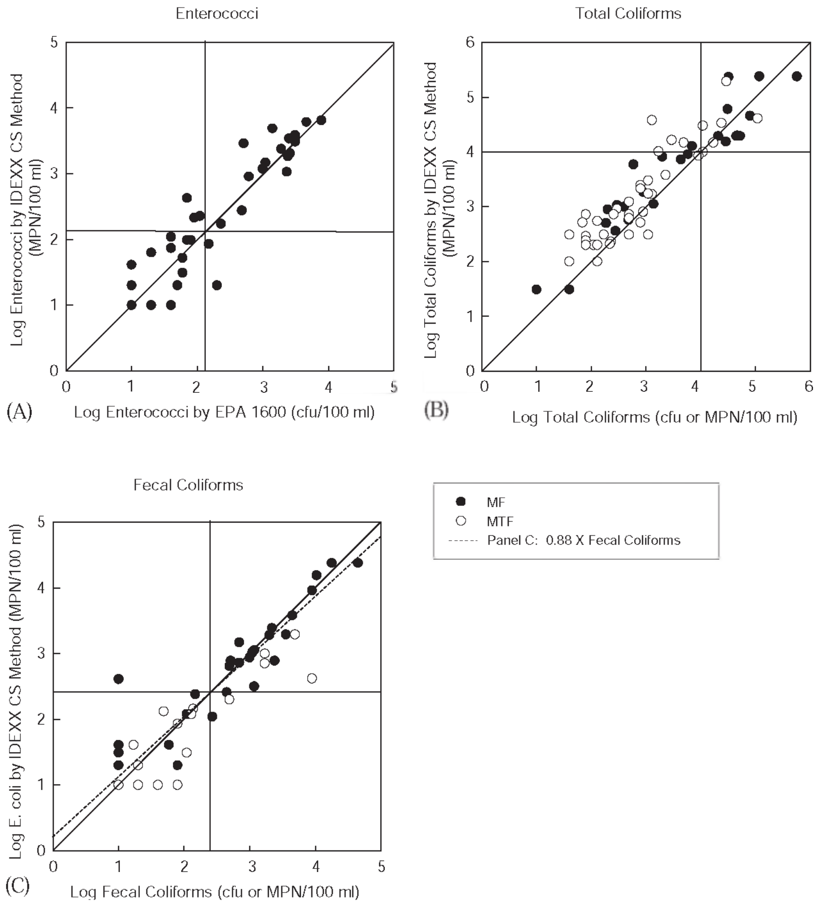

Fig. 1. MF and MTF results versus IDEXX CS kit results for EC (A), total coliforms (B), and fecal coliforms (C). Diagonal lines represent one-to-one relationship. Horizontal and vertical lines are State of California standards. Dotted line in (C) is the regression line ($0.88 \times$ fecal coliforms).

Table 2
Median ratio and associated 95% confidence interval of the ratio between results from MF or MTF and CS kits

Ratio	Median	Lower limit	Upper limit
Total coliforms			
MF/CS	0.62	0.40	1.29
MTF/CS	0.55	0.36	0.79
Fecal coliforms			
MF/CS	1.00	0.76	1.18
MTF/CS	2.00	1.00	2.51
Enterococci			
MF (EPA 1600)/CS (Laboratory 1)[a]	1.75	0.50	2.50
MF (EPA 1600)/CS (Laboratory 2)[a]	0.67	0.11	1.00

[a] Laboratory effects significant.

Table 3
Threshold agreement between methods[a]

	MF or MTF < 400	MF or MTF > 400
Fecal coliforms		
CS < 400	55	6
CS > 400	2	37
	MF or MTF < 10,000	MF or MTF > 10,000
Total coliforms		
CS < 10,000	64	0
CS > 10,000	5	31
	MF or MTF < 104	MF or MTF > 104
Enterococci		
CS < 104	38	4
CS > 104	6	52

MF: membrane filtration; MTF: multiple tube fermentation; CS: chromogenic substrate kits made by IDEXX Laboratories, Inc.

[a] Numbers represent the percent of samples within each category. All thresholds are single sample State of California standards (e.g. 104 for enterococci) and are reported in cfu or MPN/100 mL.

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.

4. Discussion

Our general finding that the IDEXX CS methods yielded comparable results to MF and MTF is consistent with that of several previous researchers [2–9]. 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 comparability of our results over a large concentration range, and in runoff-affected samples where biochemical complexity is likely to be great, 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 almost twice that for both MF and MTF methods (Fig. 1), consistent with results reported by Palmer et al. [9] and Pisciotta et al. [13]. It has been suggested that the IDEXX CS method has the potential to yield false positives with members of the *Vibrio* and *Aeromonas* spp. in subtropical waters [11,13,17], which could explain the higher values.

We also found lower fecal coliform values using CS, which is consistent with the specificity of the Colilert and Colilert-18 kits to measurement of *E. coli*, rather than to all fecal coliforms. We limited our comparison to methods which enumerate FC because State of California marine water quality standards are based on FC, and municipalities that presently use the IDEXX CS methods compare it to that standard. However, freshwater standards around the country, including California's, are specific to *E. coli*. Further studies to assess whether CS provides similar results to MF and MTF methods that are specific for *E. coli* are warranted.

Regression analysis between CS results and MF/MTF results suggests that *E. coli* constituted 88% of the FC in this study (Fig. 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 CS methods should be increased by 20% when comparing results to a fecal coliform standard. The United States Environmental Protection Agency, however, has suggested the use of a new *E. coli* criteria of 63% of the FC density [18]. The results of this study suggest that the former recommendations would both cause more conservative regulation of recreational waters than is currently enforced with the FC standards. However, 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/or geographically specific. This suggests the need for additional study of this conversion factor.

5. Conclusions

- The IDEXX CS methods generally yielded comparable results to MF and MTF methods in samples taken during the time of high stormwater runoff, with a wide range of indicator bacteria densities.
- TC results by IDEXX CS were 1.6 to 1.8 times higher than those for MF and MTF methods.
- IDEXX CS versus MF and MTF results suggested that 88% of FC were *E. coli*, though further work to determine an *E. coli* to FC conversion factor is warranted.
- When examined using a threshold-based analysis, 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 (TC, FC, and EC).

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