IDEXX Summary 6J

Topic: Colilert® and Enterolert® correlate well with MF and MTF in beach water

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Source: American Society of Microbiologists Annual Convention

Date: May, 2001

Report Highlights:

- The study concludes that Colilert and Enterolert present suitable alternatives to MF and MTF for monitoring beach water quality.
- Beach water quality at 79 different sites was measured during periods of high urban runoff.
- Samples varied widely in bacterial concentration gradients, ranging from 10 - 1.1 x 10^6 cfu/100 ml
- The study found 90 to 95% agreement with respect to the State of California's Beach Water Quality Standards between IDEXX methods and MF and MTF for total coliforms, *E. coli* and enterococci.
- The correlation between results obtained using Colilert and Enterolert vs. MF and MTF was high (0.91-0.92) for total coliforms, *E. coli* and enterococci.
- The comparability of results over these large ranges, particularly at a time when interference is likely to be greatest, provides more assurance of comparability in all environmental samples.

ABSTRACT

Two methods, membrane filtration (MF) and multiple tube fermentation (MTF), have been used for decades to monitor indicator bacteria levels in beach water samples. Other methods, involving the use of chromogenic substrate (CS) technology, have increasingly been used for monitoring beach water quality. Numerous studies have compared results between MF and MTF, but only a few have assessed the comparability between these standard methods and CS methods. The few studies that have been conducted have generally found high comparability between CS methods and standard methods, but have been conducted during dry weather. As part of a larger regional monitoring study of shoreline water quality that involved more than 20 labs in Southern California, we performed a comparison of MF, MTF, and CS methods (using kits manufactured by Idexx Laboratories, Inc.) during a storm event. We collected samples along the shoreline of the Southern California Bight after a heavy rain to compare results for total coliforms, fecal coliforms (*E. coli*), and enterococci. For the 79 sites sampled, the correlations between the CS methods (Idexx kits) and the standard methods (MF and MTF) were high ($r = 0.91 - 0.92$) for all three indicators. Correlations between indicators were high regardless of whether the samples were taken along a beach or near a storm drain. None of the comparisons between indicators were found to be significantly different using the t-test ($p < 0.05$). We found 90 to 95% agreement between methods with respect to California's Beach Water Quality Standards for all three indicators. Although comparability was high, there were systematic differences noted between the methods. These results are consistent with previous cross-laboratory intercalibration studies, and expand upon previous studies by sampling a variety of locations during a period of high urban runoff, thereby allowing us to sample a wide range of bacterial concentrations. Our results indicate that CS methods (as performed using Colilert® and Enterolert®) provide comparable results to MF and MTF methods during periods of heavy runoff, and may be suitable alternatives for monitoring beach water quality.

INTRODUCTION

Coastal waters are an important economic and recreational resource that is influenced by human activities. Treated wastewater discharges, and non-point-source industrial inputs and surface runoff all affect coastal water quality and create the impetus for extensive water quality monitoring programs. 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 bacteria most commonly used as indicators of fecal contamination are total coliforms, fecal coliforms, *Escherichia coli (E. coli),* and enterococci.

Three methods are commonly used to quantify bacterial densities, membrane filtration (MF), multiple tube fermentation (MTF), and chromogenic substrate (CS) techniques. These methods differ in (1) the speed of results, from 18 to 96 h depending upon the indicator and test method used; and (2) costs for training personnel, analysis time, reagents, and supplies. If these methods were to produce comparable results, then the fastest, least expensive method would be preferred.

Numerous studies have compared results between MF and MTF, but only a few have assessed comparability between these methods and the newer CS method (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.* 1999). These studies, conducted mostly under dry-weather conditions, have generally found high comparability between CS and the other methods. Our study expands upon previous studies by sampling a variety of locations during a period of high urban runoff, thereby allowing us to sample a wide range of bacterial concentrations. The bacterial concentration gradients we measured were large, ranging from 10–1,100,000 cfu or MPN/100 ml, with the upper end of the range for each bacterial indicator exceeding State standards by at least 100 fold. The comparability of results over these large ranges, particularly at a time when interferences are likely to be greatest, provides more assurance of comparability in all environmental samples.

Methods

Samples were collected from 79 sites along the Southern California Bight (SCB) coastline on February 22, 2000, which was one day after a storm produced from 1.1 to 3.0 inches of rain over the entire region in sufficient quantities to induce flow of the major freshwater outlets into the ocean. The sample sites were selected using a stratified random sampling design, stratified by open beach (31 sites) and sites located within 100 meters of a freshwater outlet (48 sites). All samples were collected in ankle-deep water on an incoming wave (see photo) just prior to receding, with the sampler positioned downstream from the bottle and the mouth of the bottle facing into the current.

Samples were split and analyzed using both the Idexx kits and the methods used as the standard operating procedure by the six laboratories that participated in the study. Standard methods used included 9221B, C and E, 9222B and D, 9230B and C in *Standard Methods for the Examination of Water and Wastewater*, APHA, AWWA, WEF, 18th Edition, 1995, and EPA Method 1600 (for enterococci) (APHA 1995). Not all laboratories used both methods on all samples, yielding 75 analyses for total coliforms, 51 analyses for fecal coliforms (the Idexx method, Colilert, is specific for *E. coli*), and 48 analyses for enterococci.

The bacterial densities were compared between methods using both paired t-tests and Pearson correlation coefficients, after log transformation. These analyses were conducted using all data, as well as by method type and sample site type. The comparisons were also conducted categorically by assessing the consistency of sample classification with respect to the State of California's State Beach Water Quality Standards (10,000 cfu or MPN/100 ml for total coliforms, 400 cfu or MPN/100 ml for fecal coliforms, and 104 cfu or MPN/100 ml for enterococci).

Results

The correlation between results obtained using the Idexx kits and Standard Methods was high (0.91-0.92) for all indicators (Table 1, Figure 1). Except for the comparison of fecal coliforms/*E. coli* by MTF, correlations for individual standard methods also exceeded 0.91. Similarly, correlations between methods were high regardless of whether the samples were collected on open beaches or near freshwater outlets. However, the correlation was somewhat lower (0.84) for fecal coliforms at freshwater outlets. None of the comparisons between indicators were found to be significantly different using the t-test (Table 2).

We found 90 to 95% agreement with respect to the State of California's Beach Water Quality Standards between methods for all three indicators in the categorical analysis (Table 3). The greatest agreement occurred for total coliforms, with 95% agreement; the 5% of samples that disagreed exceeded the standard for the Idexx method while meeting the standard for the laboratory's conventional method. An 8% disagreement rate was found for fecal coliforms, 6% demonstrating higher results for the Standard Method and 2% demonstrating higher results for the Idexx method. Enterococci results showed the widest variation, with 10% of the samples evenly spread between Idexx being higher and lower than the Standard Methods.

Conclusions

- The CS method (using the Idexx Labs products, Colilert® and Enterolert®) yielded comparable results to Membrane Filtration and Multiple Tube Fermentation
- These findings are consistent with previous cross-laboratory intercalibration studies (Leecaster *et al.* 2000, Noble *et al.* 2001) as well as previous within-laboratory split sample studies, and were performed over a wide range of concentrations, and salinities.
- Although comparability was high, some systematic differences were noted between the results from the Idexx method and MF and MTF. Total coliform results using the Idexx method were slightly higher than for the other two methods (Figure 1, Table

2), consistent with results reported by Palmer *et al.* (1993). Enterococci results from Idexx kits are also generally higher (Figure 1), a trend that was observed in one previous study (Eckner 1998).

- A more systematic difference found in the comparison of the two types of methods was the lower fecal coliform values recorded by the IDEXX method, Colilert® (Figure 1, Table 2). This difference reflects the fact that the IDEXX method is specific for measurement of *E. coli*, which is a subset of fecal coliforms. We found a 93% slope to regression between fecal coliforms and *E. coli*.

FIGURE 1. Standard method results versus Idexx results for each indicator. Diagonal lines represent one-to-one relationship. Horizontal and vertical lines at threshold values.

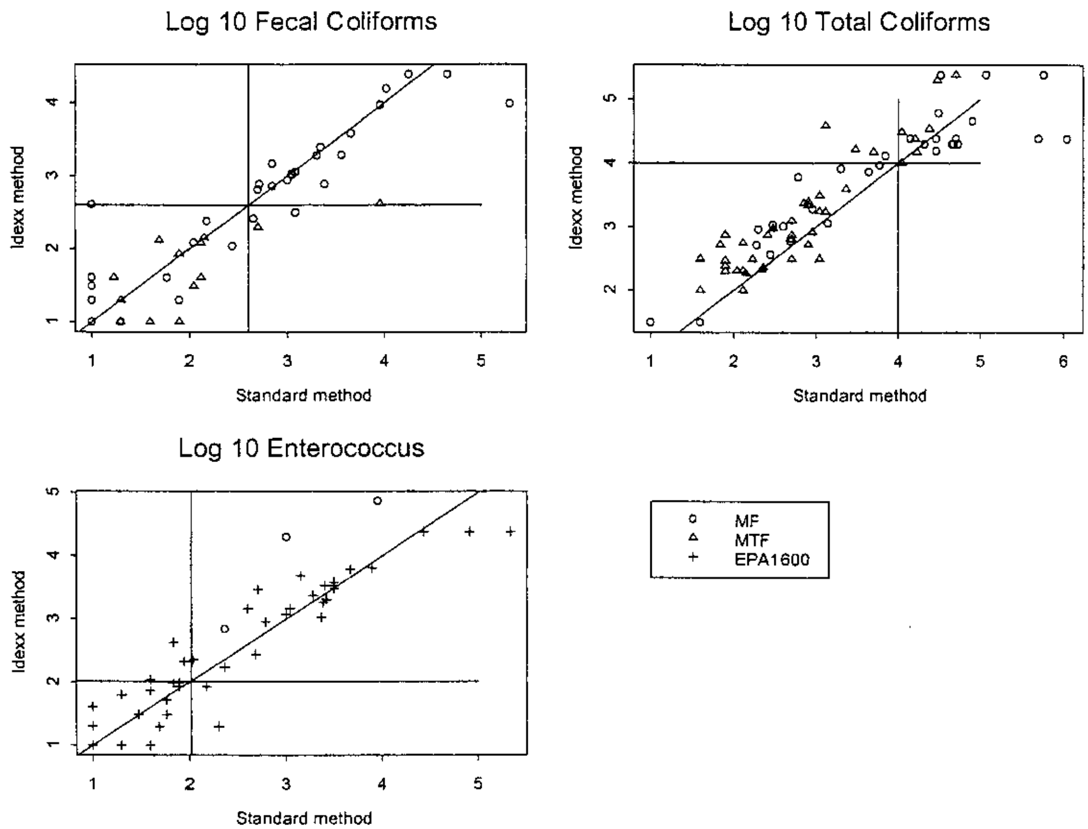

FIGURE 2. Regression comparison of fecal coliforms and *E.coli* counts.

Regression of E.coli and Fecal Coliform s

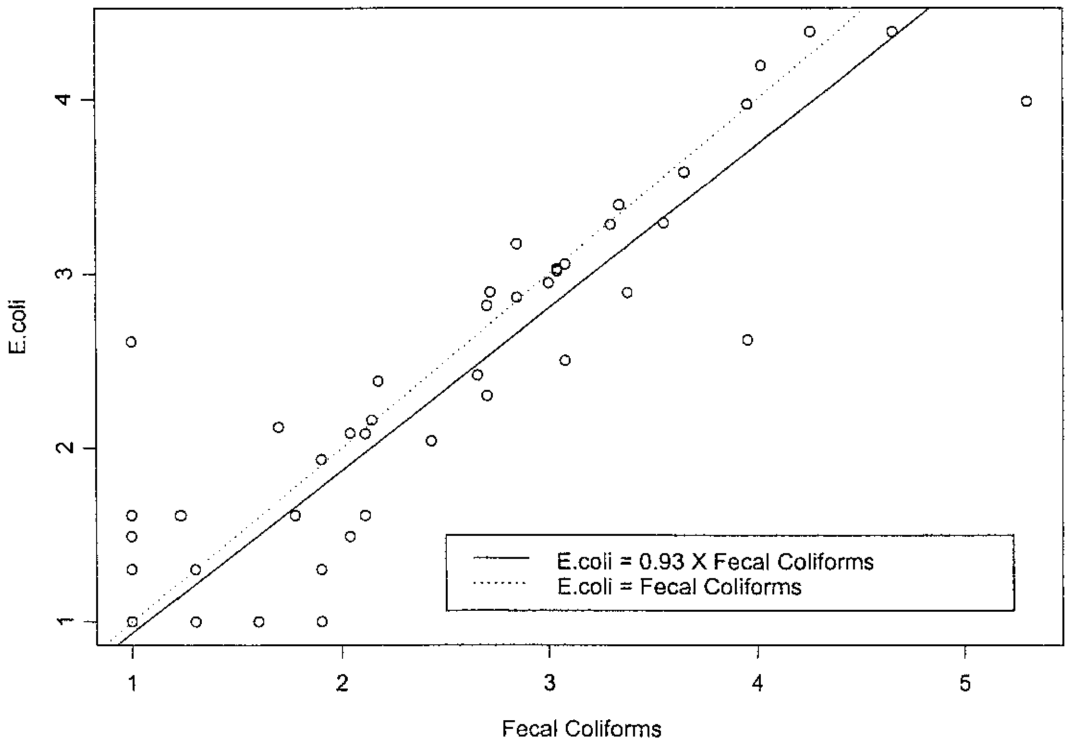

TABLE 1. Correlation between Idexx methods and other methods. Results are for overall, segregated by method and segregated by sample site type.

	Fecal Coliforms	Total Coliforms	Enterococci
Overall	0.91	0.91	0.92
Membrane Filtration	0.92	0.92	0.93
Multiple Tube	0.79	0.91	NA
EPA 1600	NA	NA	0.94
Beaches	0.95	0.92	0.92
Outlets	0.84	0.92	0.93

TABLE 2. P-value for paired t-test between methods.

Indicator	**P – value**
Fecal Coliforms	0.27
Total Coliforms	0.35
Enterococci	0.45

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

	FECAL COLIFORMS	
	Standard Method < 400	Standard Method > 400
Idexx < 400	55	6
Idexx > 400	2	37
	TOTAL COLIFORMS	
	Standard Method < 10,000	Standard Method > 10,000
Idexx < 10,000	64	0
Idexx > 10,000	5	31
	ENTEROCOCCI	
	Standard Method < 104	Standard Method > 104
Idexx < 104	38	4
Idexx > 104	6	52