# COMPARISON OF BEACH BACTERIAL WATER QUALITY INDICATOR MEASUREMENT METHODS

RACHEL T. NOBLE[1*], STEPHEN B. WEISBERG[2], MOLLY K. LEECASTER[3]
CHARLES D. MCGEE[4], KERRY RITTER[2], KATHY O. WALKER[5], AND PATRICIA M. VAINIK[6]

[1]*University of North Carolina at Chapel Hill, Institute for Marine Sciences, Morehead City, NC, USA;*
[2]*Southern California Coastal Water Research Project, Westminster, CA, USA;*
[3]*Idaho National Engineering and Environmental Laboratories, Idaho Falls, ID, USA;*
[4]*Orange County Sanitation District, Fountain Valley, CA, USA;* [5]*Sanitation Districts of Los Angeles County, Carson, CA, USA;* [6]*City of San Diego Metropolitan Wastewater Department, San Diego, CA, USA;*
[*]*(author for correspondence, e-mail: rtnoble@email.unc.edu)*

**Abstract.** Three methods (membrane filtration, multiple tube fermentation, and chromogenic substrate technology kits manufactured by IDEXX Laboratories, Inc.) are routinely used to measure indicator bacteria for beach water quality. To assess comparability of these methods, quantify within-laboratory variability for each method, and place that variability into context of variability among laboratories using the same method, 22 southern California laboratories participated in a series of intercalibration exercises. Each laboratory processed three to five replicates from thirteen samples, with total coliforms, fecal coliforms or enterococci measured depending on the sample. Results were generally comparable among methods, though membrane filtration appeared to underestimate the other two methods for fecal coliforms, possibly due to clumping. Variability was greatest for the multiple tube fermentation method. For all three methods, within laboratory variability was greater than among laboratories variability.

**Keywords**: microbiology, intercalibration, variability, bacterial indicators, beach water quality

## 1. Introduction

Coastal beaches are the subject of extensive water quality monitoring to detect fecal contamination from human activities, such as wastewater discharge, industrial input, and surface runoff. Included in many of these monitoring programs is measurement of indicator bacteria, such as total coliforms, fecal coliforms, and enterococci. While indicator bacteria are not necessarily pathogenic, they are found abundantly in human wastes, where pathogenic organisms may exist. Bacterial indicators are used in preference to direct tests for pathogens because bacteria indicator measurements are less expensive and correlate with the incidence of illness in swimmers (Cabelli, 1983; Haile *et al.*, 1999).

Indicator bacteria have historically been measured using either membrane filtration (MF) or multiple tube fermentation (MTF), though chromogenic substrate methods, such as those manufactured by IDEXX Laboratories, Inc. (IDEXX), have recently been gaining popularity. The three methods each are based upon measuring different products of bacterial growth. MF enumerates bacterial colonies on a specific growth substrate. MTF measures metabolic activity as determined by fermentation and the production of gas. Chromogenic methods measure the ability of organisms to metabolize a specific labeled substrate, thereby releasing a chromogen. These differences in analytical endpoint provide the potential for differing results among methods.

*Environmental Monitoring and Assessment* **81**: 301–312, 2003.

There have been a number of studies comparing the response of MF and MTF, but only a few studies have compared these methods to IDEXX (Edberg *et al*., 1990; Stasiak and Cheng, 1991; Green *et al*., 1997; Eckner, 1998; Francy and Darner 2000). No study has quantified response among all three methods, nor placed differences among methods into context of variability among laboratories that use the same methods. Furthermore, no study has compared measurement precision among the three methods. IDEXX kits have the advantages of being less expensive and faster than the historically used methods, but these advantages are of little value if the results produced by IDEXX are not comparable to that from the historic methods.

Here we present a series of intercalibration studies that were conducted among 22 southern California laboratories. We use these studies to assess comparability of results among the three bacterial indicator measurement methods, quantify within laboratory variability for each method and place that variability into context of variability among laboratories using the same method.

## 2. Methods

Thirteen experiments were conducted on five separate occasions (Table 1). The first set of experiments involved quantification of total coliforms in transport media at three bacterial indicator concentrations. The second set of experiments involved quantification of fecal coliforms [or *Escherichia coli (E. coli)*, to which the IDEXX method, Colilert®, is specific] in transport media at three bacterial indicator concentrations. The third set of experiments involved quantification of enterococci in transport media at three bacterial indicator concentrations. The fourth set of experiments involved quantification of total coliforms and fecal coliforms (or *E. coli*) at a single concentration in seawater and fecal coliforms (or *E. coli*) in transport medium. The fifth set of experiments involved quantification of fecal coliforms (or *E. coli*) from natural seawater spiked with treated wastewater effluent (Table 1).

Ten of the thirteen experiments were performed by seeding 24 hour-old stock cultures of *E. coli* (ATCC 75922) or *Enterococcus faecalis* (ATCC 29212) into 10-liter carboys of NYSDH-1 transport medium (Toombs and Conner 1980). Transport media was prepared prior to the day of the experiment in two-liter volumes and sterilized. Carboys were sterilized separately. Bacteria were added to the transport media and mixed for twenty minutes on a magnetic mixer prior to dispensing the first sample. Targeted concentrations were 100–1,000 (low), 1,000–10,000 (medium) and 10,000–100,000 bacteria/100 mL (high, Table 1). The amount of stock culture necessary to achieve the target concentrations was based on MF analyses from the preceding day.

In experiments 5 and 10, stock cultures of *E. coli* (ATCC 75922) were used to inoculate natural seawater collected from Palos Verdes, California. In experiment

**Table 1**. Median bacterial indicator count and standard deviation in each experiment. Numbers with the same letter code are not significantly different. MF = Membrane Filtration; and MTF = Multiple Tube Fermentation.

| *Experiment Number* | *Date* | *Indicator* | | *Matrix* | *Method* | *Median Count* | *Standard Deviation* |
|---|---|---|---|---|---|---|---|
| 1 | 04/21/98 | Fecal Coliforms | Bacterial Culture | Transport Media | MF | 306[b] | 55[a] |
| | | | | | MTF | 967[a] | 431[a] |
| | | | | | IDEXX | 996[ab] | 1,131[a] |
| 2 | 04/21/98 | Fecal Coliforms | Bacterial Culture | Transport Media | MF | 340[b] | 115[b] |
| | | | | | MTF | 2,610[a] | 1,652[a] |
| | | | | | IDEXX | 3,964[a] | 1,626[ab] |
| 3 | 04/21/98 | Fecal Coliforms | Bacterial Culture | Transport Media | MF | 65,400[b] | 9,422[a] |
| | | | | | MTF | 173,750[a] | 45,066[a] |
| | | | | | IDEXX | 160,000[ab] | 35,777[a] |
| 4 | 06/09/98 | Fecal Coliforms | Bacterial Culture | Seawater | MF | 696[b] | 125[a] |
| | | | | | MTF | 1,945[a] | 1,306[b] |
| | | | | | IDEXX | 1,601[ab] | 407[a] |
| 5 | 06/09/98 | Fecal Coliforms | Bacterial Culture | Transport Media | MF | 848[b] | 124[b] |
| | | | | | MTF | 1,928[a] | 2,454[a] |
| | | | | | IDEXX | 1,528[ab] | 379[ab] |
| 6 | 06/23/98 | Fecal Coliforms | Effluent Wastewater | Seawater | MF | 764[a] | 195[a] |
| | | | | | MTF | 1,093[a] | 1,389[a] |
| 7 | 03/31/98 | Total Coliforms | Bacterial Culture | Transport Media | MF | 179[a] | 74[a] |
| | | | | | MTF | 265[a] | 231[a] |
| | | | | | IDEXX | 172[a] | 51[a] |
| 8 | 03/31/98 | Total Coliforms | Bacterial Culture | Transport Media | MF | 1,797[a] | 280[a] |
| | | | | | MTF | 2,088[a] | 1,638[a] |
| | | | | | IDEXX | 1,613[a] | 318[a] |
| 9 | 03/31/98 | Total Coliforms | Bacterial Culture | Transport Media | MF | 21,920[a] | 3,098[a] |
| | | | | | MTF | 19,564[a] | 13,061[a] |
| | | | | | IDEXX | 16,680[a] | 6,202[a] |
| 10 | 06/09/98 | Total Coliforms | Bacterial Culture | Seawater | MF | 1,676[a] | 230[a] |
| | | | | | MTF | 2,617[a] | 2,325[b] |
| | | | | | IDEXX | 1,601[a] | 47[a] |
| 11 | 04/28/98 | Enterococci | Bacterial Culture | Transport Media | MF | 177[a] | 29[a] |
| | | | | | MTF | 246[a] | 164[a] |
| | | | | | IDEXX | 164[a] | 39[a] |
| 12 | 04/28/98 | Enterococci | Bacterial Culture | Transport Media | MF | 2,600[a] | 773[a] |
| | | | | | MTF | 2,540[a] | 1,792[a] |
| | | | | | IDEXX | 1,967[a] | 506[a] |
| 13 | 04/28/98 | Enterococci | Bacterial Culture | Transport Media | MF | 28,571[a] | 10,260[a] |
| | | | | | MTF | 41,600[a] | 23,249[a] |
| | | | | | IDEXX | 18,429[a] | 3,742[a] |

6, filtered primary-treated wastewater from the Orange County Sanitation District Plant #1 was added to natural seawater (collected from Newport Beach, California, Table 1). Before inoculating the seawater sample, the primary treated wastewater was filtered through Whatman Grade 415 filter paper to remove fine particulates.

On the morning of each experiment, the contents of the carboys were aliquotted into subsamples, which were packed in ice and distributed to the participating laboratories. All laboratories began their analyses at the same time, approximately five hours after the stock sample was prepared. The originating laboratory analyzed the first and last sample dispensed from each carboy by MF and MTF to validate homogeneity of samples aliquotted from the carboy.

Each laboratory used its own standard operating procedures. Methods used by participants included 9221B, C and E, 9222B and D, 9230B and C in Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WEF, 19th edition, 1995 and EPA method 1600 (APHA 1995). Colilert® and Enterolert™ (IDEXX Laboratories, Inc, Westbrook, ME) were used in both 15-tube MTF format (only one labortory) and 51 well Quanti-Tray® format. Three to five replicates were performed for each indicator at each concentration. Several laboratories used more than one analytical method, which resulted in more than 22 sets of results for some experiments.

To test the hypothesis that the median within-laboratory values were the same among methods, we performed an ANOVA on ranks, separately for each experiment (Conover and Iman, 1981). A Bonferoni adjustment to significance level was used to account for pair-wise testing of methods. Similar analyses were performed to test for difference in within-laboratory variance among methods.

To assess uncertainty for individual laboratories reporting counts based on a single sample, 60% and 95% confidence limits were estimated using data from all laboratories, pooled across experiments. After log transforming the data, least squares regression was used to model the within laboratory variances as a function of within laboratory means. Confidence intervals were then back-transformed to original scale. Separate regressions were performed for each indicator.

## 3. Results

Comparability of bacterial densities measured among methods was indicator specific (Figure 1; Table 1). For enterococci and total coliforms, we saw no significant differences among methods in any of the individual experiments, though we did observe that MTF results were higher in five of the seven experiments. We also observed that IDEXX yielded the lowest median count in all three of the experiments conducted with enterococci. For fecal coliforms, MF produced the lowest counts in all six tests, and in several experiments the median count was less than 50% of the other methods. MF results were significantly lower than MTF results in many of the individual tests, but differed significantly from results from IDEXX only in experiment 2.

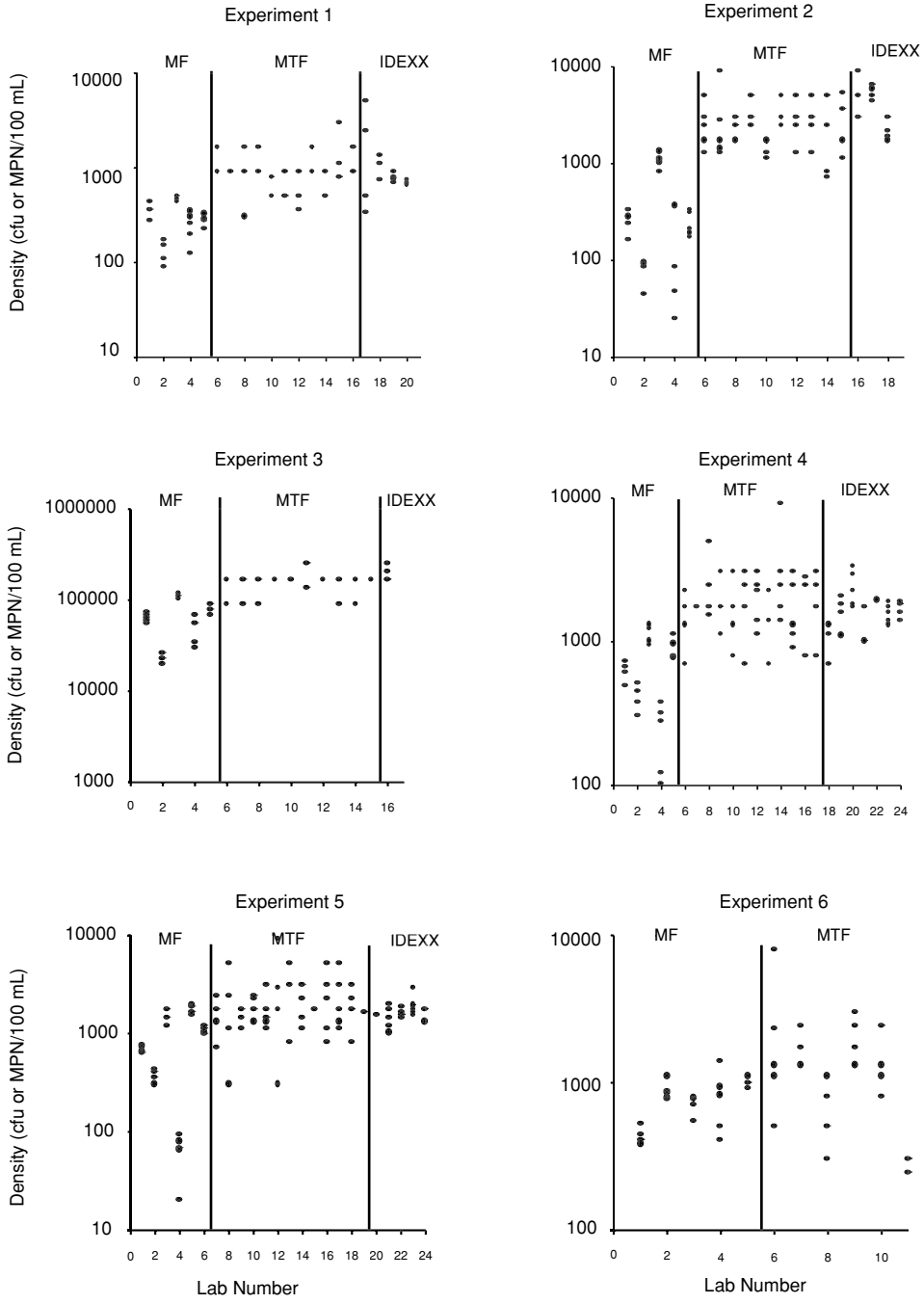


**Figure 1**. Log-transformed bacterial density vs. Laboratory (Lab) number. Laboratory numbering was random and arranged by method. Experiments 1–6 are for Fecal Coliforms. MF = Membrane Filtration Method, MTF = Multiple Tube Fermentation Method, IDEXX = Colilert® or Enterolert™.

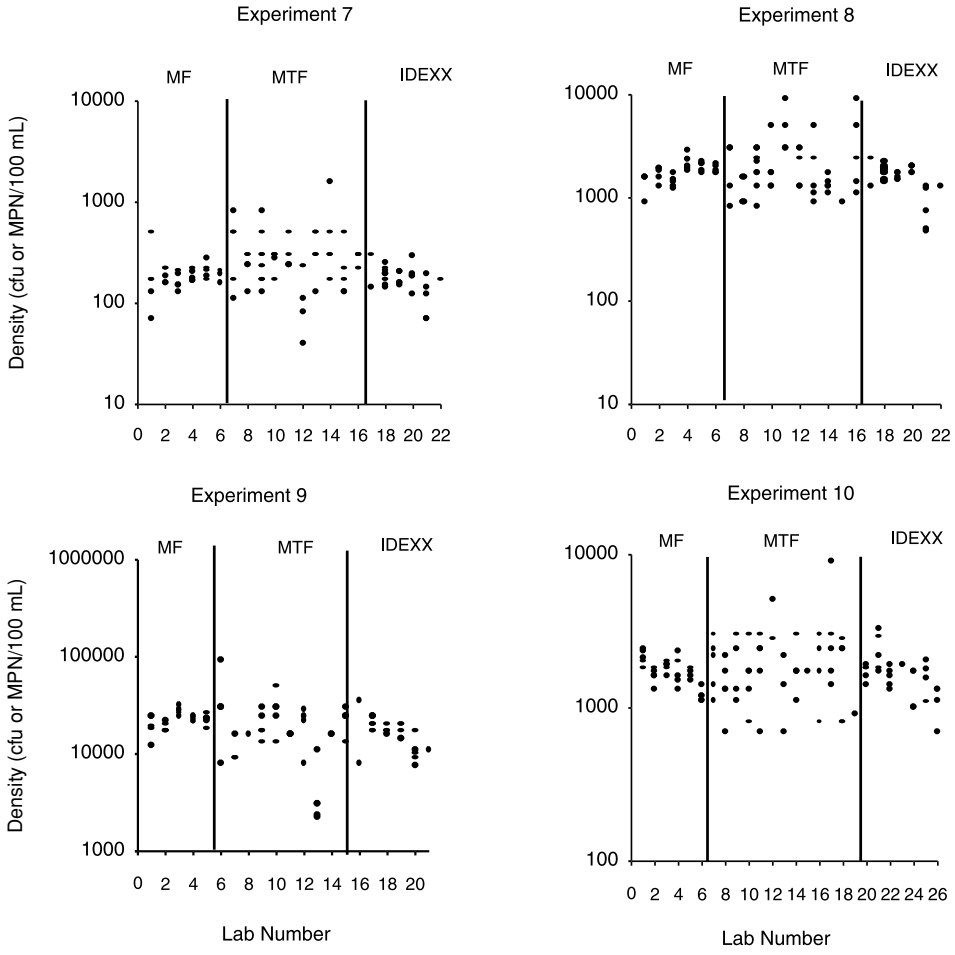


**Figure 1**—*Continued*. Log-transformed bacterial density vs. Laboratory (Lab) number. Laboratory numbering was random and arranged by method. Experiments 7–10 are for Total Coliforms.

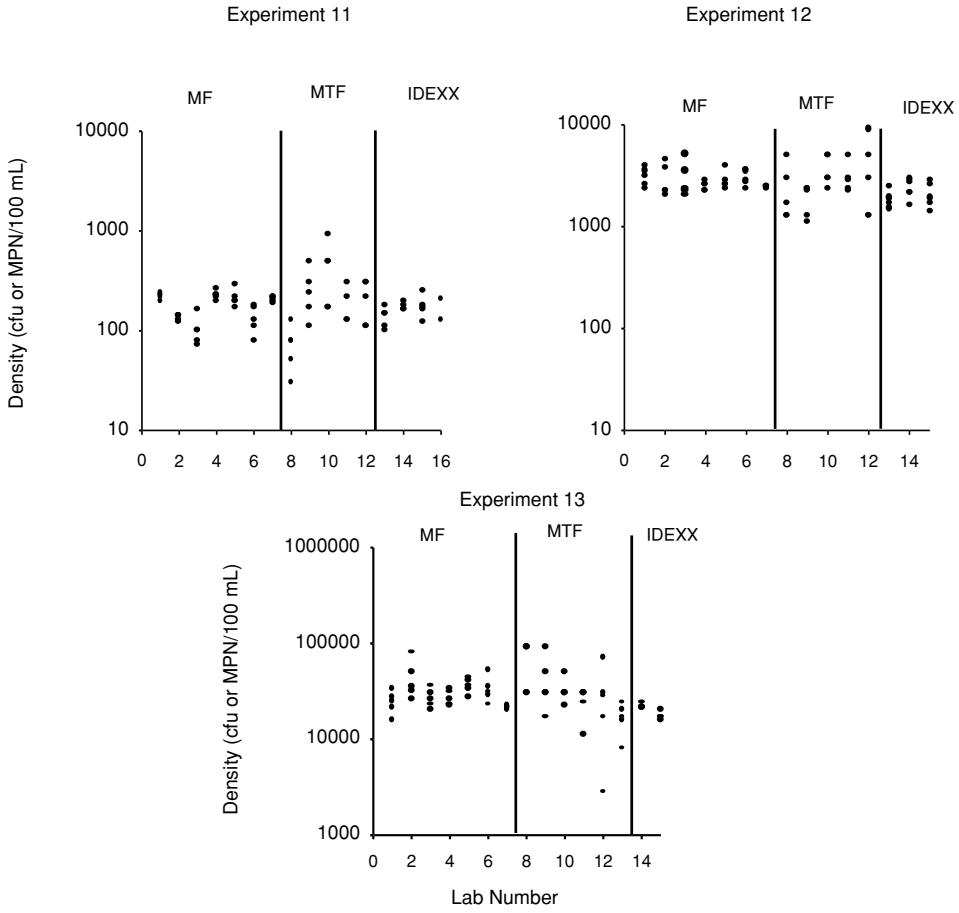


**Figure 1**—*Continued*. Log-transformed bacterial density vs. Laboratory (Lab) number. Laboratory numbering was random and arranged by method. Experiments 11–13 are for Enterococci.

Variability was consistently highest for MTF, and in several cases was almost an order of magnitude higher (Table 1). The variability associated with MF was generally a little lower than that with IDEXX, but not significantly different in any of the individual experiments. The coefficient of variation appeared to be independent of which bacterial indicator was measured.

The confidence interval for MTF was about twice as large as for the other two methods (Table 2). In most cases, the upper 95% confidence interval was more than twice the threshold value. The confidence intervals are all asymmetric around the threshold, reflecting the lognormal distribution of the data. Also, the confidence intervals presented in Table 2 are all specific to the threshold concentration, as the data displayed a significant variance: mean ratio.

**Table 2**. Upper and lower confidence limits around California's AB411 single-sample thresholds. AB411 thresholds: total coliforms 10,000 cfu or MPN/100 mL, fecal coliforms 400 cfu or MPN/100 ml, enterococci 104 cfu or MPN/100 mL.

| | | Lower 95% CI | Lower 60% CI | Upper 60% CI | Upper 95% CI |
|---|---|---|---|---|---|
| Total coliforms | MTF | 3,482 | 5,901 | 16,946 | 28,717 |
| | MF | 7,947 | 8,915 | 11,218 | 12,583 |
| | IDEXX | 4,339 | 6,587 | 15,181 | 23,048 |
| Fecal coliforms | MTF | 122 | 221 | 724 | 1310 |
| | MF | 188 | 274 | 584 | 851 |
| | IDEXX | 164 | 256 | 625 | 976 |
| Enterococci | MTF | 36 | 61 | 176 | 299 |
| | MF | 70 | 86 | 126 | 153 |
| | IDEXX | 63 | 81 | 134 | 173 |

## 4. Discussion

Several recent initiatives, including California State Assembly Bill 411, the federal Beaches Environmental Assessment, Closure, and Health (BEACH) Act and the World Health/USEPA Expert Consultation of Safety of Recreational Waters, have been catalysts for increased beach monitoring and greater consistency in standards on which to base public health warning decisions. One price for increased consistency, though, can be loss in flexibility. For instance, California, which has the most beach monitoring in the United States (Schiff *et al*., 2001), recently mandated that warnings be based on any sample that exceeds a single sample standard, whereas agencies previously had flexibility to collect confirmation samples. Examination of data from Los Angeles County reveals that a majority of the single sample standard exceedences for the five-year period between 1995 and 2000 (Schiff *et al*., In Press) were within measurement error for MTF. Conversely, there were

similar numbers of measurements that were less than the standard, but within the lower confidence bounds. These findings suggest there is a great deal of uncertainty associated with warnings based on a single sample standard.

The magnitude of our variability estimates, as well as our finding of higher variability for MTF, is consistent with that of previous studies (Fleisher, 1990). MTF is a most probable number technique, which is a statistical estimate based on the percentage of test tubes eliciting a positive response to the presence of bacteria. As the estimate is based on a binomial distribution, its variance is primarily a function of the number of tubes used. IDEXX methods are also based on a most probable number technique, but they employ a prepackaged 51-well tray that is logistically easier to use than test tubes. As a result of the greater number of wells, this method yielded a variance similar to that of MF.

Although our variability estimates were similar to that of previous studies, our finding of lower fecal coliform values using MF differed from previous studies which generally have found comparable results for MF and MTF. It also differs from our finding of similar results for total coliforms and enterococci using the two methods. Several laboratories reported filtration difficulty and colony growth that was extremely patchy, suggesting clumping of the bacteria. This could be an artifact of our using a laboratory strain inoculated into transport media. To investigate this possibility, we used a seawater matrix in experiment four and effluent as an inoculant in experiment six. We saw the same pattern of lower MF values in experiment four, but method differences were not apparent when using effluent as the inoculant. While use of a laboratory strain might increase the likelihood of clumping, discussions with our participating laboratories revealed that they loosely follow the standard method protocol which calls for shaking the sample at least 25 times to enhance homogeneity, instead typically shaking the sample only about five times. As a plating method, MF is more susceptible to clumping and our results emphasize the need for strict adherence to quality control guidelines regarding shaking and sample dilution.

Many environmental assessments require compilation of data from multiple laboratories, either to extend temporal records for trends assessment or to enhance geographic scale to accomplish regional/national assessments. Such data compilations assume a degree of comparability among laboratories, even though analytical personnel, methods, and instrumentation may vary. Our study suggests that differences among methods are small relative to inherent measurement error, though our results with fecal coliform suggest that this could be of concern if laboratory procedures are not followed precisely. We also found that differences among laboratories using the same methods were generally small and less than the normal variability encountered using a single method in a single laboratory (Figure 1). Overall, the increase in variability among measurements from pooled data was only about 30% higher than that obtained using a single analytical method performed at a single laboratory.

Our study also included participation by volunteer monitoring organizations which are becoming an increasingly large component of beach monitoring in some areas. The volunteer organizations involved in this study produced data comparable to that of the certified professional laboratories. While the volunteers involved in our study were more experienced than most, having conducted their own monitoring activities for many years and having benefited from EPA-sponsored training, our study demonstrates that data from properly trained volunteer organizations can be an equal addition to beach quality assessments.

The use of IDEXX kits has become increasingly popular in the past few years because they are less expensive and require less formal training than historically used methods. The use of IDEXX kits requires minimal space and equipment, making them ideal for volunteer organizations or for agencies without microbiology laboratories. Our study is among the first to compare this method to others and is the first test to quantify its variability. We found it to be an acceptable, and perhaps preferable, method. The median values were similar to that produced by MTF, without evidence of the clumping that seemed to compromise the MF measurements. Moreover, measurement variability was considerably less than MTF, which can be partially attributed to the use of the Quanti-Tray® 51-well format compared to the MTF procedure which typically involves a 15-tube format. Measurement variability for IDEXX was comparable to that of MF. While these findings support the use of IDEXX for marine water quality testing, they are not comprehensive. The bacteria measured in all but one of our experiments were laboratory strains, with no background bacteria to compete or interfere with the analyses. While we saw little difference in results between our experiments using laboratory cultures and wastewater effluent as inoculant, our experiments were also conducted on samples that contained low levels of suspended solids, which can potentially interfere with colony growth. Side-by-side testing of samples from the natural environment, particularly during high turbidity conditions, is a logical next step in evaluating these methods.

## Acknowledgements

The authors thank all of the participants in the study who spent laboratory time and also attended the many meetings in preparation for the exercises; Algalita Marine Research Foundation, Aliso Water Management Authority and Southeast Regional Reclamation Authority, Aquatic Bioassay and Consulting Laboratories, City of Long Beach Department of Health & Human Services, City of Oceanside, City of Oxnard, City of Los Angeles Environmental Monitoring Division, City of San Diego, City of Santa Barbara, City of Ventura, Encina Wastewater Authority, Goleta Sanitation District, Instituto de Investigaciones Oceanalogicas (UABC), Los Angeles County Department of Health Services, County Sanitation Districts of Los Angeles County, Orange County Public Health Laboratory, Orange County Sanitation District, San Diego County Department of Environmental Health, San

Elijo Joint Powers Authority, Santa Barbara Health Care Services, Southern California Marine Institute, and Surfrider Foundation. We especially thank the County Sanitation Districts of Los Angeles County and the Orange County Sanitation District for preparing the samples.

## References

American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*: 1995, 18th ed, Eaton, A.D., Clesceri, L.S., and Greenberg, A.E. (eds.), Washington, D.C.

APHA. See American Public Health Association.

Conover, W.J., and Iman, R.L.: 1981, 'Rank Transform as a Bridge Between Parametric and Nonparametric Statistics,' *The American Statistician* **35**, 125.

Cheung, W.H.S., Chang, K.C.K., and Hung, R.P.S.:1991, "Variations in microbial indicator densities in beach waters and health-related assessment of bathing water quality,' *Epidemiol. Infect*. **106**, 329–344.

Eckner, K.F.: 1998, 'Comparison of membrane filtration and multiple-tube fermentation by the Colilert and Enterolert methods for detection of waterborne coliform bacteria, Escherichia coli, and enterococci used in drinking and bathing water quality monitoring in southern Sweden,' *Appl. Environ. Microbiol*. **64**, 3079–3083.

Edberg, S.C., Allen, M.J. Smith, D.B. and Kriz, N.J.: 1990, 'Enumeration of total coliforms and Escherichia coli from source water by the defined substrate technology,' *Appl. Environ. Microbiol*. B, 366–369.

Fleisher, J.M.: 1990, 'The effects of measurement error on previously reported mathematical relationships between indicator organism density and swimming-associated illness: a quantitative estimate of the resulting bias,' *Internat. J. of Epidemiol*. **19**, 1100–1106.

Francy, D.S. and Darner, R.A.: 2000, 'Comparison of methods for determining Escherichia coli concentrations in recreational waters' *Wat. Res*. **24**, 2770–2778.

Green, B.L., Clausen, E.M. and Litsky, W.: 1977, 'Two-temperature membrane filter method for enumerating fecal coliform bacteria from chlorinated effluents', *Appl. Environ. Microbiol*. **33**, 1259–1264.

Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides and Wang, G.: 1999, 'The health effects of swimming in ocean water contaminated by storm drain runoff,' *Epidemiol*. **10**, 355–363.

Messer, J.W., and Dufour, A.P.: 1998, 'A rapid, specific membrane filtration procedure for enumeration of enterococci in recreational water,' *Appl. Environ. Microbiol*. **64**, 678–680.

Natural Resources Defense Council.: 1998, 'Testing the waters, Has your vacation beach cleaned up its act?' Vol. 8. p. 145. New York, NY.

Schiff, K.C., Weisberg, S.B. and Dorsey, J.H.: 2001, 'Microbiological monitoring of marine recreational waters in southern California,' *Environ. Management*. **27**:149–157.

Schiff, K.C., Morton, J. and Weisberg, S.B.: In press, 'Retrospective evaluation of shoreline water quality along Santa Monica Bay beaches,' *Mar. Env. Res*.

Stasiak, M.C., and Cheng, S.H.: 1991, 'Coliform detection using membrane filtration and Colilert, and *E. coli* detection using nutrient AGAR Plus MUG and EC Plus MUG,' pp. 741–748 in: Proceedings of the Water Quality Technology Conference, Orlando. American Water Works Association, Denver, CO.

Toombs, R.W., and Conner, D.A.: 1980, 'Proficiency test sample media for single and mixed pure cultures of water pollution indicator bacteria,' *Appl. Environ. Microbiol.* **40**, 883–887.