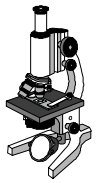


Comparison of Bacterial Indicator Measurements Among Southern California Marine Monitoring

Charles D. McGee[1], Molly K. Leecaster, Patricia M. Vainik[2], Rachel T. Noble[3], Kathy O. Walker[5], and Stephen B. Weisberg

ABSTRACT

Recent initiatives to develop regional/national assessments of beach quality require consolidation of bacteriological data across multiple laboratories. In southern California, 22 laboratories routinely measure bacterial indicators of fecal contamination using several methods. To assess data comparability, each of these labs quantified total coliforms, fecal coliforms or *E. coli*, and enterococci density from thirteen common samples. Three sources of variability (among laboratories, among analytical methods and within laboratory) were also quantified and compared. The average difference among methods was less than 6%. The average difference among laboratories was less than 2%. The greatest source of variability was among replicates within individual laboratories. Combining data from all laboratories using different methods increased variability by only about 30% over that which would be expected if a single laboratory using a single method generated all of the data.

INTRODUCTION

Coastal waters are an important economic and recreational resource that is influenced by human activities. Treated wastewater discharges, industrial inputs, and surface runoff all affect coastal water quality and create the impetus for extensive water quality monitoring programs. An important criterion for assessing the potential health risk of recreational waters to swimmers is the density of bacteria associated with fecal contamination. The bacteria most commonly used as indicators of fecal contamination are total coliforms, fecal coliforms, *Escherichia coli (E. coli),* and enterococci. Although indicator bacteria do not necessarily cause illness, they are abundant in human waste where pathogenic organisms, such as viruses and parasites, are also likely to exist. Bacterial indicators are measured instead of pathogenic organisms because the indicators occur in much larger numbers and can be measured with faster, less expensive methods than the pathogens of concern.

Nationwide, tens of thousands of marine water samples are analyzed annually for indicator bacteria (Natural Resources Defense Council 1998). Most of the analyses are part of sampling programs that are independently planned and implemented by local or county public health departments, or by Publicly Owned Treatment Works (POTWs) fulfilling federal, state and regional monitoring requirements specified in their permit to discharge wastewater into waters of the United States. In southern California alone, over 20 agencies regularly monitor near-shore water quality (Schiff *et al. 1998),* but the data are rarely combined to provide estimates or comparisons of conditions on a regional scale.

Several recent initiatives require the merger of data at regional and national levels. These initiatives, which reflect public desire for a more comprehensive assessment of beach water quality, include California Assembly Bill 411; USEPA's Beaches Environmental Assessment, Closure, and Health (BEACH) program; and the World Health/USEPA Expert Consultation of Safety of Recreational Waters. One concern that arises when

[1]Orange County Sanitation District, P.O. 8127, Fountain Valley, CA 92728
[2]City of San Diego, Metro Wastewater Dept., 4918 N. Harbor Dr., Rm. 201, San Diego, CA 92106
[3]Wrigley Institute of Environmental Studies, USC Wrigley Institute for Environ. Studies, Univ. of Southern Calif., Los Angeles, CA 90089-0371
[4]Sanitation Districts of Los Angeles County, 24501 S. Figueroa St., Carson CA 90745

consolidating data from independent programs is that the numerous laboratories which perform the analyses use different analytical methods. Standard enumeration methods for the isolation of viable bacteria from environmental samples include membrane filtration (MF) and multiple tube fermentation (MTF). Each of these enumeration formats can also be used with more than one type of media. For example, the MTF method of enumerating fecal coliform can be performed using EC or A-1 media. Enumeration using chromogenic substrate media, media that can detect enzymes produced by specific bacteria or groups of bacteria, are also available and currently being used by several monitoring agencies.

The consistency in response among methods has rarely been quantified. A few studies have compared response between pairs of methods (Eckner 1998, Stasiak and Cheng 1991, Edberg *et al*. 1990, Green *et al.* 1997) and one study examined among-laboratory variability in marine applications (Messer and Dufour 1998). No study has quantified among-method variability for the three methods (MTF, MF and chromogenic substrate kits), nor has any study placed among-method variability within the context of variability among laboratories that use the same methods. California's Environmental Laboratory Accreditation Program (ELAP) attempts to address comparability among laboratories by establishing acceptance criteria for specific test methods, but the program does not rigorously quantify inter-method or inter-laboratory variability. Within-laboratory variability between methods has been assessed on a limited basis when a laboratory demonstrates method comparability in preparation for switching from one analytical method to another.

This study examined comparability of data generated by 22 southern California laboratories when quantifying total coliforms, fecal coliforms (or *E. coli*), and enterococci densities in common samples. Participants included 12 wastewater discharger agencies, five public health departments, three volunteer organizations, one private consulting laboratory and one university laboratory (Table 1). The study assessed among laboratory, among analytical method and within laboratory variability. The additional variability introduced by pooling data from different monitoring programs using different methodologies was also quantified and placed within the context of natural variability occurring within a single laboratory program.

METHODS

Five intercalibration exercises were conducted. The first three exercises involved quantification of total coliforms, fecal coliforms (or *E. coli*) and enterococci in the transport medium. Each of the exercises used three concentrations of the bacterial indicator. The fourth exercise 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 final exercise involved quantification of a single concentration of fecal coliforms (or *E. coli*) in seawater.

TABLE 1. Laboratories participating in interlaboratory comparison study.

Laboratory	Methods Used
Algalita Marine Research Foundation	Colilert®
Aliso Water Management Authority and Southeast Regional Reclamation Authority	MF, MTF
Aquatic Bioassay and Consulting Laboratories	MTF
City of Long Beach Department of Health & Human Services	MF, MTF
City of Oceanside	MTF
City of Oxnard	MTF
City of Los Angeles Environmental Monitoring Division	MF, Colilert®
City of San Diego	MF, MTF
City of Santa Barbara	MTF, Enterolert®
City of Ventura	MTF
Encina Wastewater Authority	MF
Goleta Sanitation District	MTF
Instituto de Investigaciones Oceanalogicas (UABC)	MTF
Los Angeles County Department of Health Services	MF, MTF, Colilert®, Enterolert®
County Sanitation Districts of Los Angeles County	MF, MTF
Orange County Public Health Laboratory	MTF, Colilert®
Orange County Sanitation District	MF, MTF, Colilert®, Enterolert®
San Diego County Department of Environmental Health	MTF
San Elijo Joint Powers Authority	MTF
Santa Barbara Health Care Services	Colilert®, Enterolert®
Southern California Marine Institute	Colilert®
Surfrider Foundation	Colilert®, Enterolert®

In the first three exercises, samples were prepared by seeding 24 hour-old stock cultures of *E. coli* (ATCC 75922) or, *Streptococcus 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 was added to the transport media and mixed for twenty minutes on a magnetic mixer prior to dispensing the first sample. Targeted seeding densities were 100, 1,000 and 10,000-bacteria/100 mL. Amount of stock culture necessary to achieve the target densities was based on MF analyses begun the preceding day.

In the fourth exercise, *E. coli* was added to both seawater and transport medium. In the final exercise, filtered primary wastewater from the Orange County Sanitation District Plant #1 was added to seawater. Primary wastewater was filtered through Whatman Grade 415 filter paper. To increase homogeneity among aliquots, the seawater was filtered through a sand filter to remove large particulates.

Samples were readied by 8:00 AM, packed in ice, and distributed in time for all laboratories to begin their analyses by 1:00 PM the same day. The originating laboratory analyzed the first and last sample dispensed from each carboy by MF and MTF procedures in order to validate the homogeneity of bacteria in the carboy. Analyses were begun soon after the last sample was collected from the carboy and again four hours later. Each laboratory was allowed to use 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. Colilert® and Enterolert® (Idexx Laboratories, Inc, Westbrook, ME) kits were used in both 15-tube MTF format and 51 well Quantitray® format. Three to five replicates for each indicator at each density were required. Several laboratories used more than one analytical method, which resulted in more than 22 analytical results reported in some data sets.

Log transformed bacterial density measurements were compared among laboratories and among methods using a nested ANOVA model. Multiple comparisons were performed using Tukey's method, with alpha set to an overall experimental error rate of 0.05. Three components of variance (among-replicate variance within individual laboratories, among-laboratory variance, and among-method variance) were estimated using the sum of squares from the nested ANOVA mode.

RESULTS

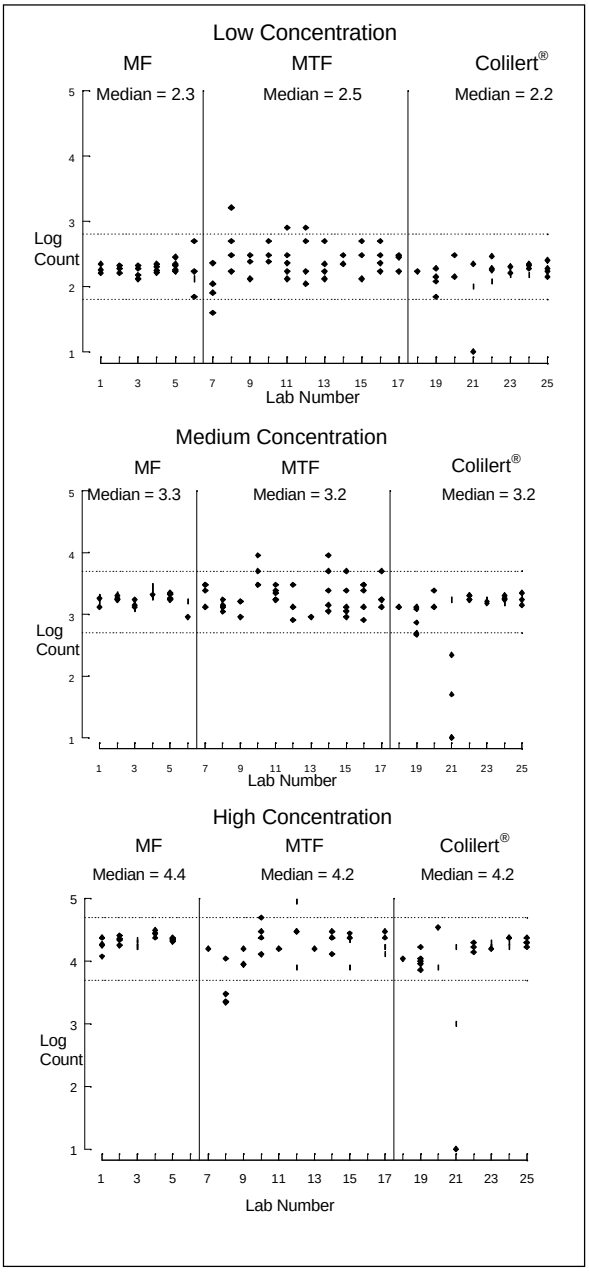
Data were highly consistent among laboratories and methods. For only 11 of the 213 analyses performed did a sample result differ by more than 0.5 log unit from the median for the test batch (Figures 1 - 5). Six of these cases were for fecal coliform recovery by MF. The remaining five cases were due to procedural errors, which were later identified and corrected. The five outlying values were removed from the data sets prior to performing statistical analysis, although they appear in the figures.

Bacterial densities differed among laboratories for seven of the 13 samples analyzed, but most of these differences were small and limited to a few laboratories. Only 7% of all of the pairwise comparisons among laboratories differed significantly, and most of these differences occurred in the early exercises. (Tables 2-4). The largest difference among laboratories was 29%, with an average difference of less than 2%. Among-laboratory differences occurred most frequently for total coliforms (10%) and least frequently for fecal coliforms (3%).

Bacterial density measurements differed significantly among analytical methods for 16 of 37 possible comparisons (43%), but the average between-method difference was less than 6% (Table 5). The largest among-method difference in any of the tests was 41%. Most of the differences among methods were due to low fecal coliform values measured by MF (Figures 2 and 4). This result remained consistent even after the six values differing by more than 0.5 log unit were removed. The *E. coli* stock culture used in these experiments was suspected to be thermophilic with a tendency to clump, which would account for the low densities reported using MF enumeration. To eliminate this potential confounding, filtered wastewater was used in place of a pure culture of *E. coli* in the final exercise. After switching to the wastewater inoculant, MF results did not differ significantly from the other two MTF enumeration formats. The only consistent difference among methods occurred for the Enterolert® method. At low densities, Enterolert® results were statistically indistinguishable from those of the other two methods, but at intermediate and higher densities, Enterolert® underestimated concentrations relative to the other two methods by 5% (Figure 3).

The largest source of variability identified in this investigation was among replicates within individual laboratories (Table 6). The MTF method yielded the greatest within-laboratory variability (Table 6), with recovery values typically ranging between one-third and three times the median value. The MF method had the

FIGURE 1. Log total coliform density from first exercise. Dashed lines are overall mean +/- 0.5 log.



smallest within-laboratory variance (Table 6), with a typical recovery range of two-thirds to 1.5 times the median value.

Among-laboratory variance was about two-thirds of the within-laboratory variance (Table 6). Similar to the pattern for the within-laboratory variability, among-laboratory variability was greatest for MTF and least for MF. Among-method variability was only about one-third of the within laboratory variance.

DISCUSSION

This investigation demonstrated that data from multiple laboratories using various analytical methods could be pooled without adding an unacceptable level of additional variability. Between-laboratory pairwise differences were generally small and improved in later interlaboratory testing efforts. The difference among methods was small, and the variability added by using multiple methods was less than the normal variability encountered using a single method in a single laboratory. Overall, the increase in variability among measurements from pooled data was approximately 30% higher than data obtained using a single analytical method performed at a single laboratory. Although none of the samples analyzed by participants in this study were environmental samples, the data suggest that a performance-based approach at multiple laboratories is acceptable for measurement of indicators of seawater contamination.

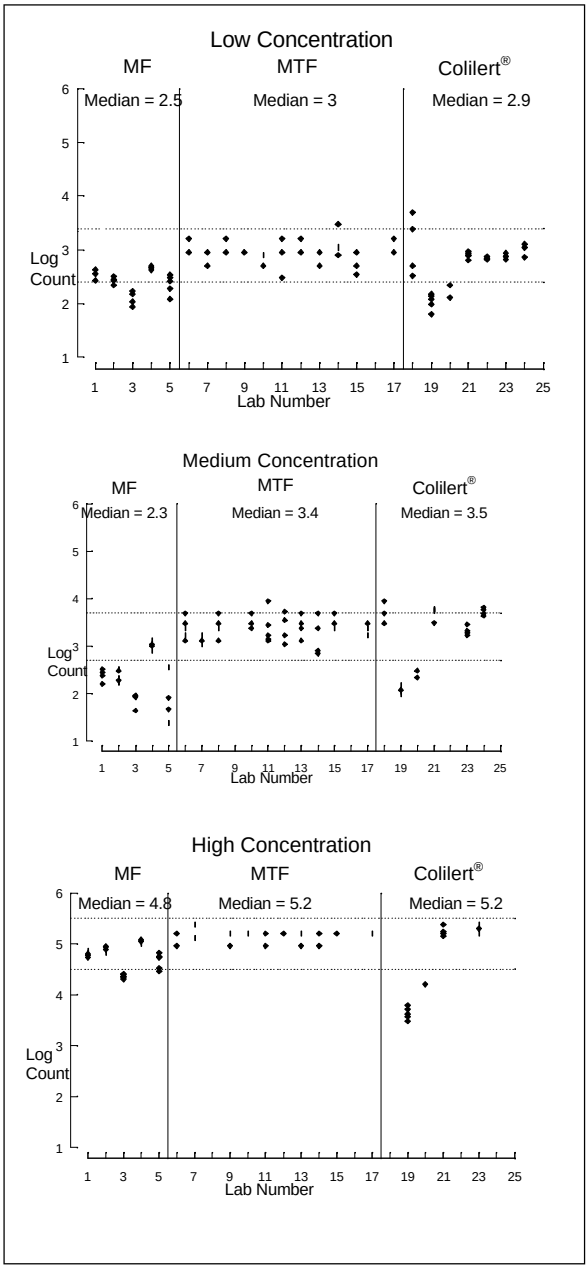
Chromogenic substrate detection methods, such as Colilert®, have not yet been approved as standard methods for marine waters by the USEPA or by the Standard Methods Committee. No significant difference was found in this study between results obtained by Colilert® and those obtained using approved standard methods for coliforms; differences in results between Enterolert® and approved methods for enterococci were small and the differences only occurred at concentrations well above California Ocean Plan standards. Data from this study also demonstrated that variability within laboratories using Colilert® was less than that for the standard MTF methods, which probably results because Colilert® is based on a 51-well format while MTF is typically performed in 15 tubes.

While these findings support the use of chromogenic substrate tests, they are not comprehensive. The bacteria measured in the first four tests were laboratory strains, with no background bacteria to compete or interfere with analyses. In informal field tests, some of the participating laboratories have noted that *Vibrio* sp. can interfere with, and lead to overestimates of, total coliforms. Also, none of the samples contained high levels of suspended solids. Low turbidity is typical in southern California in

the summer-dry season, but not always during the winter-wet season. Side-by-side testing of samples from the natural environment, particularly during high turbidity conditions, is a logical next step in evaluating these candidate methodologies.

An increasingly large component of beach monitoring in some areas is performed by volunteer organizations. One consideration in creating integrated beach assessments is whether data produced by volunteer organizations is of sufficient quality for inclusion. The volunteer organizations involved in this study produced data comparable to that of the certified professional laboratories. The volunteers involved in our study were more experienced than most, having conducted their own monitoring activities for many years. They also benefited from EPA-sponsored training and working closely with a local university. Regardless, our data show that with proper training, volunteer organizations can become full partners in developing regional beach quality assessments.

FIGURE 2. Log fecal coliform or *E. coli* density from second exercise. Dashed lines are overall mean +/-0.5 log.



LITERATURE CITED

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition. Edited by A.D. Eaton, L.S. Clesceri, and A.E. Greenberg. Washington, DC.

APHA. See American Public Health Association.

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. *Applied and Environmental Microbiology* 64:3079-3083.

Edberg, S.C., M.J. Allen, D.B. Smith, and N.J. Kriz. 1990. Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. *Applied and Environmental Microbiology* 56:366-369.

Green, B.L., E.M. Clausen, and W. Litsky. 1977. Two-temperature membrane filter method for enumerating fecal coliform bacteria from chlorinated effluents. *Applied and Environmental Microbiology* 33:1259-1264.

Messer, J.W., and A.P. Dufour. 1998. A rapid, specific membrane filtration procedure for enumeration of enterococci in recreational water. *Applied and Environmental Microbiology* 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., S.B. Weisberg, and J.H. Dorsey. 1998. Microbiological monitoring of marine recreational waters in southern California *in*: Weisberg, S.B. (ed.), Southern California Coastal Water Research Project 1997-98 Annual Report. Southern California Coastal Water Research Project. Westminster, CA.

Stasiak, M.C., and S.H., Cheng. 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 D.A. Conner. 1980. Proficiency test sample media for single and mixed pure cultures of water pollution indicator bacteria. *Applied and Environmental Microbiology* 40:883-887.

ACKNOWLEDGMENTS

The authors thank all the participants in the study (Table 1) who spent laboratory time and also attended the many meetings in preparation for the exercises. We especially thank the County Sanitation Districts of Los Angeles County and the Orange County Sanitation District for preparing the samples.

FIGURE 3. Log enterococcus density from third exercise. Dashed lines are overall mean +/- 0.5 log.

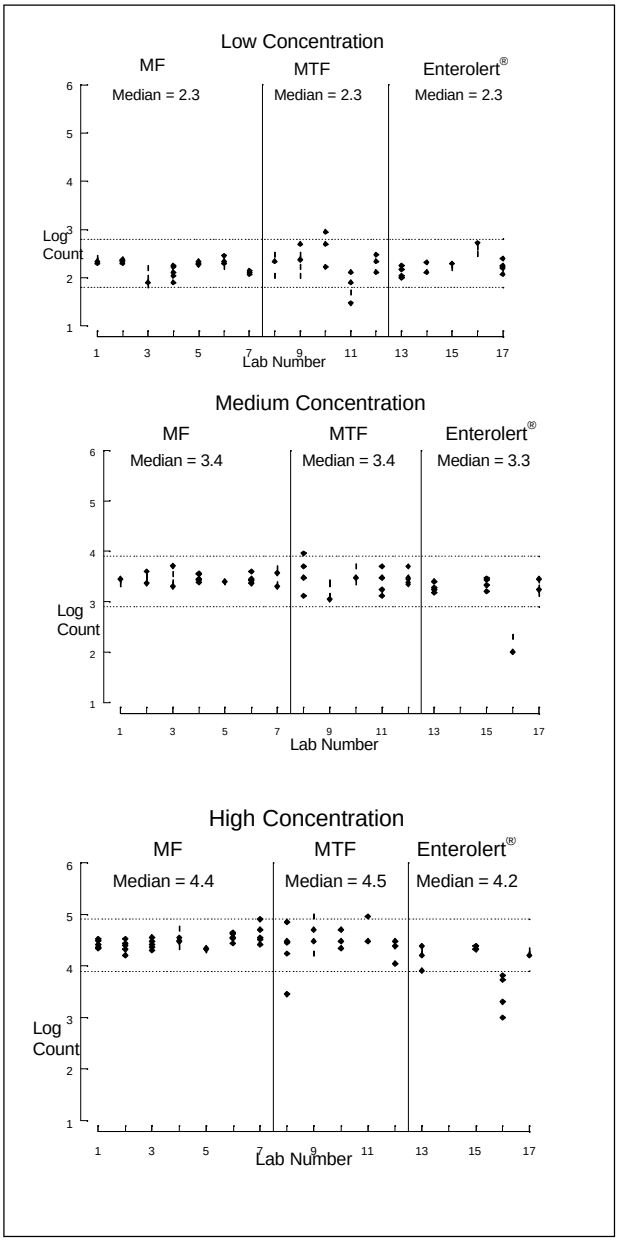


FIGURE 4. Log total coliform and fecal coliform or E. coli density from fourth exercise. Dashed lines are overall mean +/- 0.5 log.

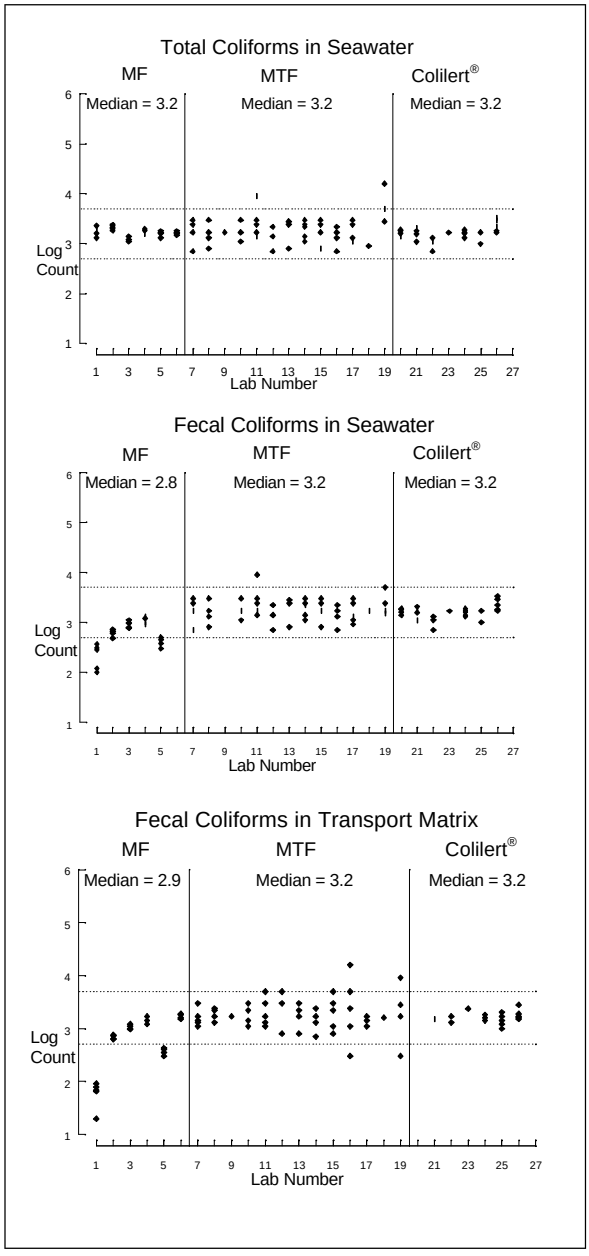


FIGURE 5. Log fecal coliform density from fifth excercise. Dashed lines are overall mean +/- 0.5 log.

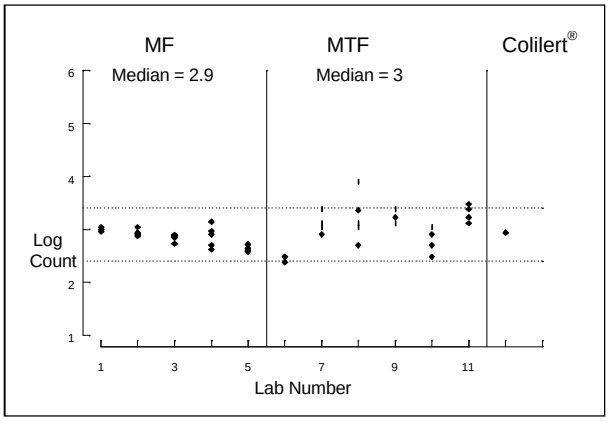


TABLE 2. Percent significant difference in fecal coliform or E. coli density between pairs of laboratories. Randomly assigned laboratory numbers are in the first row and column. NS indicates no significant difference between laboratory pairs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	NS															
3	NS	NS														
4	NS	NS	NS													
5	NS	NS	NS	NS												
6	NS	NS	NS	NS	NS											
7	NS	NS	NS	NS	**4**	NS										
8	**19**	**20**	**19**	**22**	NS	NS	**21**									
9	NS	NS	NS	NS	NS	NS	NS	**13**								
10	NS	NS	**14**	NS	NS	**14**	NS	**19**	**16**							
11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS						
12	NS	NS	NS	NS	NS	NS	NS	**23**	NS	NS	NS					
13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
14	NS	NS	NS	NS	NS	NS	NS	**20**	NS	NS	NS	NS	NS			
15	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS	NS	NS	NS	NS		
16	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
17	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS	NS	NS	NS	NS	NS	NS
18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
19	NS	NS	NS	**13**	**13**	NS	**14**	**19**	NS	**17**	**14**	**16**	NS	**18**	NS	NS
20	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
21	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
22	NS	NS	NS	NS	NS	NS	NS	**18**	NS	NS	NS	NS	NS	NS	NS	NS
23	NS	NS	NS	NS	NS	NS	NS	**18**	NS	**12**	NS	NS	NS	NS	NS	NS
24	NS	NS	NS	NS	NS	NS	NS	**20**	NS	NS	NS	NS	NS	NS	NS	NS
25	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS	NS	NS	NS	NS	NS	NS
26	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
27	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
28	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
29	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
30	**16**	**23**	**19**	**17**	**18**	**17**	**18**	**17**	**18**	**22**	**20**	**22**	**12**	**19**	NS	NS
31	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
32	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
33	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
NS															
NS	NS														
NS	NS	NS													
NS	NS	NS	NS												
NS	NS	NS	NS	NS											
NS	NS	NS	NS	NS	NS										
NS	NS	NS	NS	NS	NS	NS									
NS	NS	NS	NS	NS	NS	NS	NS								
NS	NS	NS	NS	NS	NS	NS	NS	NS							
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS						
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS					
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
18	**25**	**19**	NS	**23**	NS	**20**	**15**	NS	NS	NS	NS	NS			
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**19**		
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS

TABLE 3. Percent significant difference in total coliform density between pairs of laboratories. Randomly assigned aboratory numbers are in the first row and column. NS indicates no significant difference between laboratory pairs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	NS															
3	NS	NS														
4	NS	NS	NS													
5	NS	NS	NS	NS												
6	NS	NS	NS	NS	NS											
7	NS	NS	NS	NS	NS	NS										
8	NS	NS	NS	NS	NS	NS	NS									
9	NS	NS	NS	NS	NS	NS	NS	NS								
10	NS	NS	NS	NS	NS	NS	NS	NS	NS							
11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS						
12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS					
13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
14	**22**	NS	NS	**14**	NS	NS	NS	NS	NS	NS	NS	NS	NS			
15	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**17**		
16	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
17	**21**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**16**	NS
18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
19	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
20	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
21	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
22	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
23	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
24	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
27	**18**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
28	**20**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**15**	NS
29	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
31	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
32	NS	**21**	**18**	NS	**16**	NS	NS	NS	NS	NS	NS	NS	NS	**29**	NS	NS
33	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

TABLE 4. Percent significant difference in entrococcus density between pairs of laboratories. Randomly assigned laboratory numbers are in the first row and column. NS indicates no significant difference between laboratory pairs.

	1	2	3	5	6	8	9	10	11	12	13
2	NS										
3	NS	NS									
5	NS	NS	NS								
6	**25**	**24**	NS	**26**							
8	NS	NS	**19**	NS	NS						
9	NS	NS	NS	NS	**26**	NS					
10	NS	NS	NS	NS	**25**	NS	NS				
11	NS	NS	NS	NS	NS	NS	NS	NS			
12	NS	NS	NS	NS	NS	NS	NS	NS	NS		
13	NS	NS	NS	NS	**22**	NS	NS	NS	NS	NS	
14	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
15	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
21	NS	NS	NS	NS	**16**	NS	NS	NS	NS	NS	NS
22	NS	NS	NS	NS	**21**	NS	NS	NS	NS	NS	NS
24	NS	NS	NS	NS	**12**	**18**	NS	NS	NS	NS	NS
26	NS	NS	NS	NS	NS	**19**	NS	NS	NS	NS	NS
29	NS	NS	NS	NS	**16**	NS	NS	NS	NS	NS	NS

17	18	19	20	21	22	23	24	27	28	29	30	31	32
NS													
NS	NS												
NS	NS	NS											
NS	NS	NS	NS										
NS	NS	NS	NS	NS									
NS	NS	NS	NS	NS	NS								
NS	NS	NS	NS	NS	NS	NS							
NS	NS	NS	NS	NS	NS	NS	NS						
NS	NS	NS	NS	NS	NS	NS	NS	NS					
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			
28	NS	NS	NS	NS	NS	NS	NS	**26**	**28**	NS	NS	NS	
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

14	15	18	21	22	24	26
NS						
NS	NS					
NS	NS	NS				
NS	NS	NS	NS			
NS	NS	NS	NS	NS		
NS	NS	NS	NS	NS	NS	
NS	NS	NS	NS	NS	NS	NS

TABLE 5. Average percent difference in median log bacteria density between pairs of methods.

Total Coliform		
	MF	MTF
MTF	4%	
Colilert®	3%	2%
Fecal Coliform		
	MF	MTF
MTF	16%	
Colilert®	15%	1%
Enterococcus		
	MF	MTF
MTF	1%	
Enterolert®	3%	3%

TABLE 6. Comparison of variance components.

	MF	MTF	Colilert® Enterolert®	Pooled Over Method
Within lab variance	0.007	0.047	0.021	0.03
Among lab variance	0.01	0.04	0.01	0.02
Merged lab variance	0.01	0.077	0.027	0.05