Topic: Colilert® correlates well with mFC for enumerating fecal coliforms in wastewater

Title: Evaluation of Colilert and Enterolert Defined Substrate Methodology for Wastewater Applications

Source: Water Environment Research, Volume 74, Number 2

Author(s):   Gary P. Yakub, David A. Castric et al.

Date: March/April 2002

Report Highlights:

- Colilert results for detecting <u>fecal coliforms</u> correlated well with mFC, producing a 0.8 Pearson correlation coefficient when 40 samples were tested using a "thermotolerant coliform" protocol, which calls for incubation of samples at 44.5 °C +- 0.2 °C for 24 hours.

- In a second comparison study of 251 samples, Colilert results correlated well with mFC, (0.85 correlation coefficient). The author concluded that the 44.5 °C Colilert "thermotolerant coliform method is an excellent substitute for the enumeration of fecal coliform bacteria from treated wastewater effluents, and that the method is robust enough to be used in broad-scale comparison studies in which a number of uncontrolled variables are present."

- The study states that Defined Substrate Technology® tests "are not subject to the numerous interferences that are associated with membrane filtration and, thus, are more reproducible."

Evaluation of Colilert and Enterolert Defined Substrate Methodology for Wastewater Applications

Gary P. Yakub, David A. Castric, Kathleen L. Stadterman-Knauer, Michael J. Tobin, Mary Blazina, Tracey N. Heineman, Gim Y. Yee, Lanie Frazier

ABSTRACT: This study evaluated the utility of defined substrate methodology (DSM) for the enumeration of indicator bacteria in wastewater applications. Two commercial products, Colilert and Enterolert systems (Idexx Laboratories, Westbrook, Maine), were evaluated for variation, false-positive results, and method correlation in both surface waters and treated wastewater effluent. The DSM tests performed as well or better than the traditional methodology. The Colilert total coliform test was also evaluated for its utility in estimating fecal coliforms by incubation at 44.5 ± 0.2 °C. The modified DSM total coliform test correlated well to membrane filtration (Pearson correlation coefficient 0.8) for two different sample groups, demonstrating its utility as a screening tool to estimate fecal coliform densities. *Water Environ. Res.*, **74**, 131 (2002).

KEYWORDS: Colilert total coliform test, Enterolert enterococcus group test, defined substrate methodology, membrane filtration, thermotolerant coliforms.

Introduction

Enumeration of water quality indicator bacteria has always been a critical part of any water quality evaluation. Traditional enumeration methodologies for members of the coliform group (including fecal coliforms), such as membrane filtration (APHA et al., 1992) and multiple tube fermentation (APHA et al., 1992), are labor intensive, time consuming, require specialized training, and are quickly overwhelmed by large numbers of samples.

Idexx Laboratories (Westbrook, Maine) has developed two products for the assay of water samples: the Colilert system for the simultaneous enumeration of total coliforms and *Escherichia coli*, and the Enterolert system for the enumeration of members of the enterococcus group. Both of these systems use defined substrate methodology (DSM) to detect the presence of appropriate organisms. This methodology is based on an organism's ability to metabolize a chromogenic or fluorogenic substrate into a detectable end product. Members of the total coliform group use the enzyme, β-D-galactosidase, to convert o-nitrophenol-β-D-galactopyranoside into the yellow end product, o-nitrophenol. *Escherichia coli* use the enzyme, β-D-glucuronidase, to convert 4-methylumbelliferyl-β-D-glucuronide (MUG) into the end product, 4-methylumbelliferone, that fluoresces under long wavelength (365 nm) UV light (Park et al., 1995). Members of the enterococcus group (*Streptococcus faecalis, S. faecium, S. gallinarum*, and *S. avium*) also produce the enzyme, β-glucosidase, that catalyzes the production of 4-methylumbelliferone at 41 °C. These products greatly reduce the time, labor, and skill level needed to perform bacterial evaluations, allowing a laboratory to process more samples at a lower cost per sample (AWWARF, 1993). The U.S. Environmental Protection Agency (U.S. EPA) has approved use of the Colilert method for source waters and finished drinking waters (U.S. EPA, 1999a).

While many researchers have examined the Colilert and Enterolert DSM systems for marine and fresh surface waters (Budnick et al., 1996; Cowburn et al., 1994; and Fricker, 1997), the purpose of this study was to examine the performance of these systems for applications in wastewater, most notably for the enumeration of indicator bacteria in treated wastewater effluents. Toward this end, (1) the variance associated with the Colilert, Enterolert, and membrane filtration methodologies in three matrices (laboratory deionized water, surface water, and treated wastewater effluent) was examined; (2) the two fluorogenic methods (Colilert *E. coli* and Enterolert) for the occurrence of false-positive results were examined; (3) a protocol to apply the Colilert total coliform test to estimate fecal coliform levels by incubation at 44.5 ± 0.2 °C (referred to as "DSM thermotolerant coliforms" for the purpose of this study) was developed and evaluated; (4) the variance of the DSM thermotolerant coliform test in the three aforementioned matrices was examined; and (5) the correlation between the DSM thermotolerant coliform test and traditional fecal coliform membrane filtration using two distinct protocols was examined. Additionally, the levels of water quality bacteria in 11 different water types were examined to determine if any conclusions could be drawn between water type and indicator ratio.

Methods and Materials

Indicator Testing. All *E. coli* enumerations were performed with the Colilert system, counting fluorescent wells under 365-nm UV light after incubation at 35 ± 0.5 °C for 24 ± 2 hours. The enumerations for DSM thermotolerant coliforms were performed using the Colilert system by counting yellow-colored wells after incubation at 44.5 ± 0.2 °C for 24 ± 2 hours. Group D streptococcus bacteria (enterococcus group plus *S. bovis* and *S. equines*) were enumerated using the Enterolert system, counting fluorescent wells under 365-nm UV light after incubation at 41 ± 0.5 °C for 24 ± 2 hours. Protocols for both the Colilert and Enterolert tests are similar in that a packet of the appropriate nutrient or indicator powder is added to 100 mL of sample. After the reagent is dissolved (typically 3 to 5 minutes), the sample is poured into a sterile quantitation tray that is mechanically heat sealed into a series of large and small wells. The tray is then placed into the appropriate incubator. All enumerations were performed using Quanti-tray/2000 (Idexx Laboratories) trays, which use a most

probable number-based protocol with a quantitation range from less than 1 colony forming unit (cfu)/100 mL to 2419 cfu/100 mL without sample dilution. All lots of Colilert, Enterolert reagents (Idexx Laboratories) and Quanti-tray/2000 trays were tested to confirm sterility and product performance with the appropriate positive and negative controls prior to use. All fecal coliform analyses used for the variance and correlation studies were performed according to *Standard Methods* (APHA et al., 1992). Treated effluent samples were portioned into 20-, 10-, and 1-mL aliquots, filtered using sterile techniques, and grown on sterile m-FC media (Millipore Corp., Bedford, Massachusetts). Surface water samples were portioned into 20-, 1-, and 0.1-mL aliquots, filtered using sterile techniques, and grown on laboratory-prepared m-FC media (Becton-Dickinson Laboratories, Cockeysville, Maryland). Positive and negative controls were used to monitor sterility and product performance.

Variation Analysis. Each of the four methods of enumeration was subjected to two trials of 10 replicate analyses for each trial. Two trials for each method were performed in laboratory deionized water, a representative surface water, and treated wastewater effluent to examine the matrix contribution to variation. The data for each trial set were analyzed for mean recovery, standard deviation, 95% confidence interval, minimum and maximum value, and coefficient of variation (relative standard deviation). The average coefficient of variation for both trials in each matrix was calculated for each method and used as a measure of variation for each method in a given sample matrix.

False-Positive Analyses. Both of the fluorogenic methods were subjected to confirmation methodology to establish false-positive rates. These analyses were performed on wastewater samples, and all positive wells were selected for confirmation. For *E. coli*, positive confirmation was by gas production in EC medium (Difco Laboratories, Sparks, Maryland) after incubation at $44.5 \pm 0.2\ °C$ for 24 ± 2 hours. Because the *E. coli* positive wells had already produced a positive reaction to the Colilert MUG-based media, further growth with gas production in EC medium at $44.5 \pm 0.2\ °C$ was considered sufficient evidence of specificity for *E. coli* for the purpose of this evaluation. For group D streptococcus bacteria, initial positive confirmation was the growth of brownish black colonies with brown halos on bile esculin azide agar (Difco Laboratories) at $35 \pm 0.5\ °C$ for 24 ± 2 hours (APHA et al., 1992), verified by subjecting approximately 20% of the positive agar plates to the enzyme latex agglutination test (APHA et al., 1992). These tests were performed using the Baltimore Biological Laboratories Streoptocard enzyme latex test (Becton-Dickinson Laboratories). For each method, in both surface water and treated wastewater effluent, a minimum of 100 large wells and 100 small wells were tested. False-positive rates were calculated for each method as a function of well size and sample matrix.

Correlation Analyses. Two separate correlation studies were performed to compare the DSM thermotolerant coliform protocol to the traditional fecal coliform membrane filtration protocol. The specifics of each study are shown in Table 1.

Indicator Ratios in Different Water Types. Eleven different water types were monitored on a random basis for DSM thermotolerant coliforms, *E. coli*, and group D streptococcus bacteria levels in an attempt to determine if any conclusions could be drawn regarding indicator performance as a function of water type and use. The 11 water types included in this study were: primary and secondary effluents from a large, urban, activated-sludge wastewater treatment plant (WWTP) (approximate flow of 8×10^5

Table 1—Comparison of two separate correlation studies.

	Correlation A	Correlation B
Number of samples (n)	41	251
Sample matrix	Treated effluent	Mixed matrices[a]
Analyst	Same	Different
Laboratory facility	Same	Different
Range (CFU/100 mL)	9–265	5–17 000
Sampling protocol	Split samples[b]	Paired samples[c]

[a] Matrices include various surface waters and wastewater-contaminated waters.
[b] One sample collected, split into two for analyses.
[c] Two samples collected, analyzed individually.

m^3/d); an urban stream flowing through mostly residential–industrial land use areas; an urban river with mostly heavy industrial use; a multi-use river with mostly recreational use; a small surface water retention pond located at a major commercial landfill; a small surface water stream under the influence of acid mine drainage (approximately 4 mg/L aluminum, 200 mg/L calcium, 5 mg/L iron, 90 mg/L magnesium, and 4 mg/L manganese); primary influent and secondary effluent from a small, residential, activated-sludge WWTP (approximate flow of 4000 m^3/d); a stream flowing through mostly agricultural–livestock use areas; and a large, rural lake with mostly recreational use. Each location was analyzed using the methodologies presented in this report, and the data were compared for average indicator ratios.

Results and Discussion

In 1986, U.S. EPA released a study evaluating the effectiveness of fecal coliforms, *E. coli*, and enterococcus bacteria as indicators of water quality (U.S. EPA, 1986). In that report, U.S. EPA characterized fecal coliforms as poor indicators of water quality. While recognizing the change in status of the fecal coliform analysis, the authors believe that this analysis will remain important to the wastewater community because of the large volume of historical data that has been accumulated using this technique. Two-thirds of all states still use fecal coliforms to monitor the quality of both freshwater and marine water (U.S. EPA, 1999b).

Defined substrate methodology has been examined for its utility in drinking water (Edberg et al., 1989, and Olson et al., 1991), fresh surface waters (Olson et al., 1991, and Clark et al., 1991), brackish subtropical surface waters (Solo-Gabriel et al., 2000), marine environments (Palmer et al., 1993), recreational bathing waters (Eckner, 1998), food (Venkateswaran et al., 1996), and correlation in treated wastewater effluents (Fricker et al., 1995). The primary objective of the study was to examine the utility of the Colilert and Enterolert systems for wastewater applications.

The authors' initial hypothesis, with respect to method variation, was two-fold: (1) that the variation would be greater for membrane filtration because of physical interference at the membrane surface, the somewhat subjective nature of visual colony enumeration, and the combined interference from confluent and competing growth; and (2) that the variation within each test method would increase as the sample matrix biodiversity and physical complexity increased. Table 2 summarizes the results for each method in each matrix. These results support the hypothesis that membrane filtration will exhibit greater variability than the corresponding DSM

Table 2—Average percent coefficient of variance by method and matrix.[a,b]

Method	Treated effluent	Surface water	Laboratory deionized water
Fecal coliform[c]	31.1	27.4	28.6
Thermotolerant coliform[d]	23.6	18.4	12.9
E. coli[e]	22.7	16.8	16.9
Group d Streptococcus[f]	22.5	24.7	29.9

[a] Average of two trials, ten replicates each trial.
[b] All trials have mean recoveries greater than 20 CFU/100 mL.
[c] Standard Methods 9222D (Membrane filtration).
[d] Colilert total coliform test incubated at 44.5 ± 0.2 °C.
[e] Colilert E. coli test incubated at 35 ± 0.5 °C.
[f] Enterolert test incubated at 41 ± 0.5 °C.

test. When comparing the coefficient of variance to the fecal coliform membrane filtration and the DSM tests for E. coli and DSM thermotolerant coliforms, the DSM test variance averaged 8% lower in treated wastewater effluent, 10% lower in surface water, and 14% lower in laboratory deionized water. This suggests that there is a good deal of variance in colony growth conditions and colony enumeration for the membrane filter method that is not seen using the DSM quantitray method. Evidently, the DSM tests are not subject to the numerous interferences that are associated with membrane filtration and, thus, are more reproducible.

The second part of this study in terms of method performance was an evaluation of the false-positive results in the fluorogenic methods, a primary concern because of the regulatory environment in which these analyses are routinely conducted. For treated wastewater effluents, the results are used to assess bacterial water quality under a permitted system in which a high false-positive rate could result in unnecessary waste of disinfectant, an increase in the release of disinfection byproducts into the environment, or a violation of a discharge permit. Activated sludge treated wastewater effluent matrices have a greater biodiversity than typical surface waters, and, therefore, are believed to have a greater potential for false-positive results with the fluorogenic methods. This may be especially true for E. coli because incubation at 35 °C in a nutrient media may encourage competing growth from other species in the matrix. Other studies have demonstrated that some plant, algae, and bacteria are also capable of β-D-glucuronidase activity that has been shown to induce a false-positive result with the DSM techniques (Berger, 1994; Davies et al., 1994; and Landre et al., 1998). The elevated temperature used to incubate DSM thermotolerant coliforms, along with a lack of specificity with regard to the species present, precluded any investigation of false-positive rates for the DSM thermotolerant coliform analyses. Based on laboratory experience, it is the authors' assumption that elevated temperature would suppress most competing noncoliforms or other organisms.

Table 3 summarizes results of false-positive analyses for each DSM method as a function of well size and matrix. For the enterococcus DSM test, false-positive rates of approximately 5% were found that were consistent with the published literature (Budnick et al., 1996, and Chen et al., 1995). This supports the assumption that incubation at elevated temperature (in this case, 41 °C) precludes most competition. The E. coli DSM test showed similar false-positive rates for the surface water matrix, but ele-

vated false-positive rates for the treated wastewater effluent matrix. These data support the hypothesis that incubation at a moderate temperature (in this case, 35 °C) and in a sample matrix of rich biodiversity could produce elevated false-positive rates in the Colilert DSM E. coli test. It should be noted that further investigation still needs to be done with regard to false-positive testing for the Colilert DSM E. coli test.

To provide a more thorough investigation of the DSM E. coli test, a more comprehensive examination of the ability of the Colilert test to enumerate E. coli in wastewater is planned. Additionally, other studies have indicated difficulties with traditional gas production confirmation methodologies, most notably the finding that E. coli, in some cases, do not produce gas because of the lack or loss of the enzyme, formate-hydrogen lyase (Angles D'Auriac et al., 2000). Thus, the false-positive results obtained for E. coli should be recognized as a starting point for a more in-depth investigation of both the organism and the robustness of current confirmation methodologies. It should be noted that the rate of false-negative occurrence was not considered in this evaluation because other researchers have demonstrated it to be inconsequential for the DSM analyses (Budnick et al., 1996, and Cowburn et al., 1994).

The third part of this study involved assessing the utility of the current DSM tests to approximate conventional fecal coliforms. The hypothesis reached was that, by incubating the Colilert total coliform test at 44.5 ± 0.2 °C, the result should correlate with the traditional membrane filtration method for fecal coliforms. A somewhat more sensitive test was also expected because of the elimination of some of the interferences commonly encountered in the filtration method. For this evaluation, two different correlation studies were performed.

Correlation study A was a true split sample comparison of the two methods performed in treated wastewater effluent. This design permitted direct comparison of the alternate methods with a minimal amount of variation (Table 1). The results were log-transformed and subjected to paired t-test and Pearson linear regression analyses using Systat (V.5.2) statistical software (SPSS Inc., Evanston, Illinois). Paired t-test results indicated no statistically significant difference between the two methods (t-statistic = −1.324; corresponding probability $p = 0.2$ exceeds the confidence level of 0.05) as they were performed in study A. The Pearson linear correlation coefficient was 0.8, showing a strong linear correlation between the two methods.

Table 3—Percent false positive rate by method, matrix, and well size.[a]

Method	Treated effluent		Surface water	
	Large wells	Small wells	Large wells	Small wells
E. coli[b,c]	13.2	17.5	3.0	10.7
Group D streptococcus[d]	1.9	5.9	1.0	3.9

[a] Percentage of wells that failed confirmation analyses; minimum of 100 wells tested for each category.
[b] Colilert E. coli test incubated at 35 ± 0.5 °C.
[c] Confirmation by growth or gas in EC medium at 44.5 ± 0.2 °C after utilization of MUG media.
[d] Enterolert test incubated at 41 ± 0.5 °C.

Table 4—Indicator population ratio by water type.

Water type	n^a	Average ratio of thermotolerant coliform:E. coli: Group D streptococcus
Large wastewater primary effluent	7	13:20:1
Large wastewater secondary effluent	7	10:16:1
Urban/industrial stream	3	7:5:1
Urban/industrial river	4	19:9:1
Recreational river	6	20:8:1
Surface water retention pond/landfill	4	10:9:1
Small surface water stream/acid mine	6	70:82:1
Small wastewater primary influent	5	9:12:1
Small wastewater secondary effluent	5	49:71:1
Agricultural stream	6	5:2:1
Large recreational lake	3	1:1:1[b]

[a] Number of random samples collected.
[b] Indicator results always very low, typically less than 5 CFU/100 mL.

Correlation study B was conducted in a less controlled manner to approximate real-world sampling conditions. The two methods were performed by several different analysts at two different laboratory facilities on duplicate samples collected at the same time from the individual sources. This data set contains field samples from a variety of matrices (various surface waters and wastewater-contaminated surface water). The data were log-transformed and the Pearson correlation coefficient for this data set was 0.85, again showing that a strong linear correlation exists between these two methods. These results suggest that the DSM thermotolerant coliform method is an excellent substitute for the enumeration of fecal coliform bacteria from treated wastewater effluents, and that the method is robust enough to be used in broad-scale comparison studies in which a number of uncontrolled variables are present.

The final part of this study determined indicator bacterial population ratios in various water types. Table 4 presents the average ratios of indicator bacterial populations as a function of water type and use. Two apparent trends can be noted from these data. First, in some of the waters that were impacted by human activity (such as the wastewater treatment, the acid mine stream, and the surface water retention pond at the landfill), E. coli levels were observed to be higher than the DSM thermotolerant coliform levels in some or all of the individual samples. Because the E. coli levels are expected to be equal or lower by definition, there must be something, such as false positives, test variation, or other factors, affecting the test results.

Second, group D streptococcus levels were much closer to the E. coli levels in the rural and agricultural waters. This finding may support the view that group D streptococcus bacteria are more common in the feces of animals than in humans. Further studies should be conducted to determine if this ratio could be a useful index for estimating the relative sources of fecal pollution. It should be emphasized that these trends are based on a limited number of observations, which also demonstrates the need for additional investigations into these areas.

Conclusions

Examination of the DSM Colilert and Enterolert tests demonstrated that they performed equal to or better than membrane filtration with respect to test variation in a variety of matrices. This study was also able to demonstrate acceptable rates of false-positive results for the fluorogenic methods, with the possible exception of the Colilert E. coli test in the treated wastewater effluent matrix. As a result, a more in-depth examination of the Colilert E. coli test is planned to further evaluate its usefulness in wastewater applications. The study was also able to demonstrate the robustness of the DSM thermotolerant coliform method that was developed as an improved technique for estimating fecal coliform bacteria. Additionally, the study authors found that, for the most part, the DSM techniques provided a faster and less expensive (unpublished observations) alternative to traditional methods of bacterial enumeration.

Acknowledgments

Authors. Gary P. Yakub (microbiologist), Kathleen L. Stadterman-Knauer (environmental scientist), Michael J. Tobin, Ph.D. (laboratory manager), and Tracey N. Heineman (chemist), work in the Industrial Wastes Department of the Allegheny County Sanitary Authority. David A. Castric (microbiologist), Mary Blazina (bacteriology supervisor), Gim Y. Yee (microbiologist), and Lanie Frazier (microbiologist) work for the Division of Laboratories, Public Health Section of the Allegheny County Coroners Office, Pittsburgh. Correspondence should be addressed to Gary P. Yakub, Allegheny County Sanitary Authority, 3300 Preble Ave., Pittsburgh, PA 15233; e-mail: gyakub@alcosan.org.

References

American Public Health Association; American Water Works Association; and Water Environment Federation (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th Ed., Washington, D.C.

American Water Works Association Research Foundation (1993) Improving Bacterial Analysis While Lowering the Cost. *Research Applications. Rep. 4.* Denver, Colo.

Angles D'Auriac, M.; Roberts, H.; Shaw, T.; Sirevag, R.; Hermansen, L.F.; and Berg, J.D. (2000) Field Evaluation of a Semiautomated Method for Rapid and Simple Analysis of Recreational Water Microbiological Quality. *Appl. Environ. Microbiol.*, **66**, 4401.

Berger, S.A. (1994) Increased Protection Afforded by the Defined Substrate Technology Colilert System by its Ability to Detect Shigella β-glucuronidase. *Lett. Appl. Microbiol.*, **19**, 53.

Budnick, G.E.; Howard, R.T.; and Mayo, D.R. (1996) Evaluation of Enterolert for Enumeration of Enterococci in Recreational Waters. *Appl. Environ. Microbiol.*, **62**, 3881.

Chen, C.M.; Doherty, K.; Gu, H.; Dichter, G.; and Naqui, A. (1995) Enterolert—A Rapid Method for the Detection of Enterococcus Spp. Idexx Laboratories, Westbrook, Maine.

Clark, D.L.; Milner, B.B.; Stewart, M.H.; Wolfe, R.L.; and Olson, B.H. (1991) Comparative Study of Commercial 4-Methylumbelliferyl-β-D-Glucuronide Preparations with the *Standard Methods* Membrane Filtration Fecal Coliform Test for the Detection of *Escherichia coli* in Water Samples. *Appl. Environ. Microbiol.*, **57**, 1528.

Cowburn, J.K.; Goodall, E.J.; Fricker, E.J.; Walter, K.S.; and Fricker, C.R. (1994) A Preliminary Study of the Use of Colilert for Water Quality Monitoring. *Lett. Appl. Microbiol.*, **19**, 50.

Davies, C.M.; Apte, S.C.; Peterson, S.M.; and Stauber, J.L. (1994) Plant and Algal Interference in Bacterial β-D-Galactosidase and β-D-Glucuronidase Assays (1994). *Appl. Environ. Microbiol.*, **60**, 3959.

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 in Southern Sweden. *Appl. Environ. Microbiol.*, **64**, 3079.

Edberg, S.C.; Allen, M.J.; and Smith, D.B. (1989) National Field Evaluation of a Defined Substrate Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with Presence–Absence Techniques. *Appl. Environ. Microbiol.*, **55**, 1003.

Fricker, C.R.; Fricker, E.J.; Goodall, T.; and Cowburn, J. (1995) Quantitative Procedures for the Detection of *E. coli*, Coliforms, and Enterococci in Water, Using Quantitray and Enterolert. *Proc. Am. Water Works. Assoc. Water Qual. Technol. Conf.*, Denver, Colo.

Fricker, E.J.; Illingworth, K.S.; and Fricker, C.R. (1997) Use of Two Formulations of Colilert and Quantitray for Assessment of the Bacteriological Quality of Water. *Water Res.*, **31**, 2495.

Landre, J.P.B.; Gavriel, A.A.; and Lamb, A.J. (1998) False Positive Coliform Reaction Mediated by *Aeromonas* in the Colilert Defined Substrate Technology System. *Lett. Appl. Microbiol.*, **26**, 352.

Olson, B.H.; Clark, D.L.; Milner, B.B.; Stewart, M.H.; and Wolfe, R.L. (1991) Total Coliform Detection in Drinking Water: Comparison of Membrane Filtration with Colilert and Coliquik. *Appl. Environ. Microbiol.*, **57**, 1535.

Palmer, C.J.; Tsai, Y.L.; Lang, A.L.; and Sangermano, L.R. (1993) Evaluation of Colilert-Marine Water for Detection of Total Coliforms and *Escherichia coli* in the Marine Environment. *Appl. Environ. Microbiol.*, **59**, 786.

Park, S.J.; Lee, E.J.; Lee, D.H.; Lee, S.H.; and Kim, S.J. (1995) Spectrofluorometric Assay for Rapid Detection of Total and Fecal Coliforms from Surface Water. *Appl. Environ. Microbiol.*, **61**, 2027.

Solo-Gabriel, H.M.; Wolfert, M.A.; Desmarais, T.R.; and Palmer, C.J. (2000) Sources of *Escherichia coli* in a Coastal Subtropical Environment. *Appl. Environ. Microbiol.*, **66**, 230.

U.S. Environmental Protection Agency (1999a) In National Primary Drinking Water Regulations. *U.S. Code of Federal Regulations*, 40 CFR, Part 141, Subpart C, Section 141.21, Washington, D.C.

U.S. Environmental Protection Agency (1999b) *Action Plan for Beaches and Recreational Waters*. EPA-600/R-98-079, Office of Research & Development, Washington, D.C.

U.S. Environmental Protection Agency (1986) *Ambient Water Quality Criteria for Bacteria—1986*. EPA-440/5-84-002, Office of Air & Radiation, Washington, D.C.

Venkateswaran, K.; Murakoshi, A.; and Satake, M. (1996) Comparison of Commercially Available Kits with *Standard Methods* for the Detection of Coliforms and *Escherichia coli* in Foods. *Appl. Environ. Microbiol.*, **62**, 2236.