APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1991, p. 1535–1539 Vol. 57, No. 5
0099-2240/91/051535-05$02.00/0


# Total Coliform Detection in Drinking Water: Comparison of Membrane Filtration with Colilert and Coliquik

B. H. OLSON,[1]* D. L. CLARK,[1] B. B. MILNER,[1] M. H. STEWART,[2] AND R. L. WOLFE[2]

*Program in Social Ecology, University of California, Irvine, California 92717,[1] and Metropolitan Water District of Southern California, La Verne, California 91750[2]*

Received 23 October 1990/Accepted 27 February 1991

**The Colilert (CL) and Coliquik (CQ) systems were compared in a presence-absence format against the *Standard Methods* membrane filtration (MF) technique to determine whether differences existed in total coliform detection. Approximately 750 water samples were collected from distribution systems, covered and uncovered storage reservoirs, well sites, and the influent to drinking water treatment plants. Samples were analyzed for total coliforms and heterotrophic bacteria with MF, CL, and CQ. The agreements between CL and MF and between CQ and MF were both greater than 94.8%, which indicates that both may be acceptable methods for total coliform detection. Disagreement between the CL and CQ methods was primarily due to false-negative results. Furthermore, laboratory and field inoculation methods were compared for CL, more than 98% agreement was obtained. This finding indicates that sampling and immediate field inoculation may be an alternative to the traditional laboratory inoculation.**

The recently promulgated Coliform Rule will change the manner in which coliform testing for drinking water in the United States is conducted (9). Previously, the maximum contaminant level was based on reporting the number of coliforms per 100 ml, but is being changed to a presence-absence (P-A) form of reporting percentages of samples that are positive. This change has brought about a reexamination of traditional testing methods. Until recently, the two methods for enumerating coliform bacteria from drinking water were the most-probable-number and the membrane filtration (MF) techniques. Both of these procedures have disadvantages, including lengthy incubation times (up to 96 h for confirmation) (1), potential interference by heterotrophic plate count (HPC) bacteria (11), and difficulties in interpreting results. In addition, separate testing procedures are required to detect fecal coliforms (1). In response to these disadvantages, a new system was developed that allows for easier interpretation of results for both total coliforms and a primary fecal coliform, *Escherichia coli*, within 24 h with a reported detection sensitivity of 1 CFU/100 ml (7).

In the new methodology, two active substrates, *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG), are combined to simultaneously detect total coliforms and *E. coli*. Total coliforms produce the enzyme β-galactosidase, which hydrolyzes ONPG and thereby releases *o*-nitrophenol, which produces a yellow color. *E. coli* produces the enzyme β-glucuronidase, which hydrolyzes MUG to form a fluorescent compound. Currently, two companies, Access Analytical and Hach, are marketing rapid coliform detection systems named Colilert (CL) and Coliquik (CQ), respectively. Both of these products incorporate MUG and ONPG and can be used in a most-probable-number or P-A format.

This study was undertaken to compare the CL and CQ products with a *Standard Methods* MF method for total coliform detection. The P-A method was evaluated because the new maximum contaminant levels for drinking water are no longer based on coliform densities but on the percentages of samples that are positive. This is the first study to compare two commercial products on a variety of water sources. Further, this study was designed to compare similar products utilizing the ONPG-MUG formulation in the presence of naturally high heterotrophic bacterial counts and to compare results based on laboratory or field inoculation of water samples.

## MATERIALS AND METHODS

**Sample collection.** Water collection was carried out in accordance with the *Standard Methods* procedures (1). Samples were collected in sterile 500-ml polyethylene sampling bottles (Fisher, Pittsburgh, Pa.) containing 10% sodium thiosulfate. Before collection, the sites were flushed for 5 to 10 min and the temperature and chlorine residual concentration were recorded. Samples were transported to the laboratory on ice and analyzed within 24 h. After vigorous hand shaking of the 500-ml sample bottle, samples were analyzed for HPC bacteria and coliforms.

**System description.** Four-hundred sixty-one (62%) of the water samples were received from a distribution system located in Orange County, Calif. The water from this system was treated in a local filtration plant utilizing conventional treatment processes, including flocculation, sedimentation, filtration, and postdisinfection with chloramines. Source water to the plant is a blend of Colorado River water and state project water from Northern California. The chloramine residual of the water entering the distribution system is approximately 1.50 mg/liter. The temperature of the water in the distribution system ranges seasonally from 10 to 25°C. The water leaving the plant is maintained at a pH of approximately 8.0. An additional 55 (7.0%) samples were taken from influent water of the above-mentioned filtration plant.

One-hundred twenty-three (16.4%) of the samples were taken from an uncovered finished water reservoir located in South Orange County, Calif. The detention time of the water in this $3.76 \times 10^6$-$m^3$ reservoir is approximately 30 days. The reservoir receives conventionally treated water. The influent water is breakpoint chlorinated to remove ammonia and is

* Corresponding author.

rechlorinated upon leaving the reservoir to achieve a free chlorine residual of 1.3 to 1.5 mg/liter. The reservoir has a maximum depth of 100 feet (ca. 30.48 m).

Eighteen (2.4%) of the samples were taken from four uncovered treated water reservoirs ranging in size from 200 to 10,000 acre-feet, located in Los Angeles, Calif. All four reservoirs receive water from a Los Angeles filtration plant. The filtration plant receives its source water from the Owens Valley and treats water by using preozonation, flocculation, coagulation, and filtration. Water is chlorinated upon leaving the filtration plant and again when it leaves the reservoirs. The free chlorine residual in the water in the reservoirs ranges from 0.0 to 0.6 mg/liter. Normally the chlorine is in trace concentrations. For three of the four reservoirs, the average detention times range from 16 to 54 days. The temperature ranges seasonally from 4.0 to 25.5°C. The average pH of the water leaving the reservoirs is 8.0. The fourth reservoir has an average detention time of less than 1 day, and the average free chlorine residual in the water from this reservoir is 0.45 mg/liter. In addition to the reservoir samples, 17 (2.3%) water samples were taken from a Los Angeles distribution system that receives its water from the Los Angeles filtration plant described above. The average free chlorine residual at these sites ranges from trace levels to 0.61 mg/liter, and the seasonal temperature in the system ranges from 10 to 24°C.

Six (0.8%) of the water samples were taken from an untreated water reservoir in the Los Angeles area. The reservoir has a temperature range of 4.0 to 24.5°C.

Sixty-nine (9%) of the water samples were taken from an Orange County distribution site that sporadically receives well water, treated surface water, and a blend of these two water types. The free chlorine residual ranges from trace to 0.5 mg/liter.

**HPCs.** HPC bacteria were enumerated by the pour plate technique with tryptone-glucose agar (Difco, Detroit, Mich.) and the membrane filtration technique with R2A medium (Difco). All HPC plates were processed in duplicate. Pour plates were inverted and incubated at 35°C for 48 h, and MF-R2A plates were incubated at room temperature (20 to 23°C) for 7 days. The average number of CFU per milliliter of sample was calculated and recorded.

**Coliform MF.** Total coliforms were enumerated by the MF method with M-endo-LES agar (Difco) as described previously (1). Appropriate dilutions were performed on samples suspected to contain elevated levels of coliforms. Plates were incubated at 35°C and read at 24 h. All suspect coliforms were picked and confirmed for gas production within 48 h in lauryl tryptone broth (LTB) and brilliant green bile-lactose broth (BGB). Suspect coliforms were defined as all typical colonies exhibiting the green metallic sheen as well as the atypical colonies that were dark red mucoid with or without nucleation and/or sheen production. Confluent filters or those exhibiting colonies too numerous to count were aseptically placed into BGB and incubated at 35°C. A culture that produced gas within 24 to 48 h was considered positive for coliforms.

**P-A tests.** CL and CQ P-A tests were performed over a 1-year period from May 1989 to April 1990 in parallel with the coliform MF procedure. Each sample bottle was thoroughly shaken as previously described, and 100-ml aliquots from the 500-ml bottle were aseptically poured into two sterile wide-mouth glass reaction vessels (Fisher). The first reaction vessel received predispensed CL reagent (Access Analytical, Branford, Conn.), and the second received 2.37 g of CQ reagent (Hach Co., Loveland, Colo.). Dissolution of reagents was facilitated by vigorous hand shaking of reaction bottles. P-A coliform samples were incubated at 35°C for 24 h. A sample that changed from colorless to yellow after 24 h was recorded as positive for total coliforms. A sample exhibiting a weak color change after 24 h was incubated for an additional 4 h. If additional incubation did not yield a stronger color change to yellow, the sample was recorded as negative.

TABLE 1. General physical, chemical, and biological characteristics of water samples[a]

| Parameter | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| Total $Cl_2$ residual concn (mg/liter) | 1.1 | 0.6 | 0.0 | 1.8 |
| Temp (°C) | 22.9 | 2.3 | 10.0 | 27 |
| Atypical coliforms/100 ml[b] | 19.6 | 78.8 | 0 | 1,000 |
| Typical coliforms/100 ml[b] | <1.0 | 1.2 | 0 | 15 |
| HPC/ml (MF + R2A) | 5,244 | $2.2 \times 10^4$ | 0 | $2.4 \times 10^5$ |
| HPC/ml (pour plate) | 90 | 351 | 0 | 4,140 |

[a] $n = 749$.
[b] Excludes filters on which colonies were too numerous to count or confluent.

**Identifications.** All confirmed coliforms (gas production on LTB and BGB) and P-A samples for which there was disagreement between the MF and P-A tests were streaked for isolation onto M-endo-LES agar. Colonies were selected to represent each morphology type and streaked for isolation on R2A agar. Isolates were identified by conventional biochemical methods with API 20E (Analytab Inc, Plainview, N.Y.) test strips. Pure cultures were maintained on R2A slants at 4°C until identifications could be performed.

**Substrate specificity.** For cases in which CL or CQ were negative for total coliforms and the MF test was positive for total coliforms, attempts were made to isolate and identify coliforms from the negative P-A sample. Pure cultures of these isolated coliforms were reinoculated into the P-A test media by aseptically inoculating 24-h pure cultures into sterile reaction vessels containing 100 ml of sterile phosphate buffer (pH 7.0). Dissolution of the reagents was facilitated by vigorous hand shaking of the vessels, which were then incubated at 35°C for 24 h. A positive reaction for total coliforms was interpreted as meaning that the coliform was capable of utilizing the substrate (ONPG).

**Statistics.** The positive and negative P-A responses from the CL and CQ tests were compared against the responses from the MF test. The total agreement was determined to be equivalent to the sum of positive and negative responses that agreed with the MF results. False-positive reactions were defined as positive CL or CQ readings for which negative MF readings were obtained. False-negative reactions were defined as negative CL or CQ results that did not agree with a positive MF reaction. All responses for each method were examined and summarized against the remaining methods in $2 \times 2$ tables. Statistical analysis were conducted with Statistical Package for the Social Sciences software (SPSS $PC^+$). Statistical evaluation included the McNemar chi-square test (19). Statistical evaluations were performed at an $\alpha$ of $<0.05$. Poisson probabilities were used to confirm the probability of coliforms in false-negative samples.

## RESULTS AND DISCUSSION

**General characteristics of the system.** The data in Table 1 indicate that most samples carried a chloramine residual of

TABLE 2. Comparison of total coliform detection for the MF test versus the CL and CQ tests

| MF result | No. of samples | | | |
|---|---|---|---|---|
| | CQ[a] | | CL[b] | |
| | Positive | Negative | Positive | Negative |
| Positive | 128 | 8 | 127 | 33 |
| Negative | 3 | 432 | 0 | 589 |

[a] McNemar chi-square value, 1.46; $P > 0.05$; $n = 571$.
[b] McNemar chi-square value, 31.03; $P < 0.05$; $n = 749$.

>1 mg/liter. The data also indicate that the temperature is reflective of the western United States with a minimum of 10°C and a maximum of 27°C. Although the water temperature for certain locations drops to 4°C, no samples were collected in that range. These sampling characteristics provide two interesting findings. First, the majority of coliforms isolated on M-endo-LES agar were atypical. Second, as noted in previous studies, the membrane filtration procedure with R2A medium recovers far greater numbers of HPC than does the pour plate method (16, 18). Heterotrophic bacteria did not affect the occurrence of false-negative and false-positive results for either commercial test as determined by McNemar chi-square analysis ($\alpha > 0.05$).

**Comparison of MF and commercial preparations.** Statistical analysis of the data showed no significant difference for coliform detection between untreated and treated drinking water among the tests. Therefore, the data have been condensed by combining treated and untreated samples. The data in Table 2 indicate that there was no statistical difference between the CQ and MF techniques in detecting total coliforms in the water samples (McNemar chi-square analysis). A statistically significant difference (Table 2) was found between the CL and MF tests, indicating that the MF test was superior in detecting coliforms in the waters tested. When we compared false-positive and false-negative results of the two commercial preparations (Table 3), we found that the CL test produced more false-negative results than did the CQ test (Table 4). This is also the primary reason for lack of consistency of test results among the CL, CQ, and MF tests (Table 2). The CL test resulted in 26 false-negative and -positive errors, whereas the CQ test only accounted for 11. Thus, the total error rate for the CL test was 41% greater than that of the CQ test. The false-positive errors in the CQ and CL tests appear to be comparable; nearly equal numbers were produced by both products, whereas the CL test produced 52.9% more false-negative results than did the CQ test.

As in the case of these commercial products with the isolation of *E. coli* from false-negative samples (4), it is difficult to isolate coliforms from some false-negative samples. Coliforms were not isolated in 25 and 33% of false-negative samples for the CQ and CL tests, respectively. Coliforms were isolated in these instances from MF samples. Because it could be argued that the reason for no isolation was that no coliforms were inoculated into the test vial, Poisson probabilities for not having inoculated the test vial with a coliform were calculated for various concentrations of coliforms per volume (Tables 5 and 6). These Poisson probabilities indicate that coliforms were inoculated into CL and CQ media. The data indicate that the probability of not inoculating a coliform into the CL medium based on the concentration of coliforms in a 100-ml water sample ranges from $10^{-2}$ in the case of 1 coliform per 100 ml to $10^{-59}$ in the case of >10 coliforms per 100 ml. Similar results were obtained with the CQ medium, except that at a concentration of 1 coliform per 100 the probability dropped to 1 in 10 that a coliform was not inoculated into the medium. The data in Table 3 and 4 also indicate that confluent filters caused a greater false-negative rate for the CL test than for the CQ test. These data suggest that a proportion of coliforms do not use ONPG upon an initial inoculation for a variety of reasons, including injury, substrate specificity, and substrate sensitivity. A study by Lewis and Mak showed that it could be difficult to verify results from the CL assay after 24 h of sample incubation (15). These findings concurred with difficulties in verification of false-negative results in the CQ and CL tests in our study and also agreed in that a low number of false-positive results were obtained with the CL test.

In another investigation in which no chlorinated samples were tested, the majority of false-negative CL samples corresponded to MF filters with high background bacterial counts (17). In the present study confluent filters did not

TABLE 3. Comparison of total coliform detection with the CL and CQ tests[a]

| CQ result | No. of samples with CL result | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 108 | 23 | 131 |
| Negative | 4 | 436 | 440 |
| Total | 112 | 459 | 571 |

[a] McNemar chi-square value, 12.00; $P < 0.05$. Since the CQ product was obtained during the course of the study, only 571 samples were available for comparative analysis with the CL system.

TABLE 4. Comparison of error between false-positives and -negatives in the CL and CQ tests[a]

| Test result | No. of samples with the indicated result | | |
|---|---|---|---|
| | CL | CQ | Total |
| False-positive | 0 | 3 | 3 |
| False-negative | 26 | 8 | 34 |
| Total | 26 | 11 | 37 |

[a] McNemar chi-square value, 19.86; $P < 0.05$.

TABLE 5. Sensitivity of CL medium for the detection of total coliforms from treated and untreated water and the probability of obtaining a negative result when coliforms are present in the sample

| No. of coliforms CFU/100 ml[a] | | No. of samples | Negative ONPG reaction, % ($P$)[b] |
|---|---|---|---|
| Range | Mean | | |
| 1 | 1 | 10 | 40 ($1.83 \times 10^{-2}$) |
| 2–5 | 3 | 30 | 17 ($3.059 \times 10^{-7}$) |
| 6–10 | 8 | 5 | 80 ($1.27 \times 10^{-14}$) |
| >10 | 45 | 40 | 8 ($2.35 \times 10^{-59}$) |
| CF | 1[c] | 75 | 17 ($4.14 \times 10^{-8}$) |

[a] Determined by enumeration of coliforms with the MF method. CF, confluent filters confirmed for total coliforms by positive reaction in BGB medium.
[b] Probability of obtaining the given value based on the number of positive and negative samples and the mean concentration of coliforms.
[c] Mean concentration was determined by assuming that at least one coliform had to be present.

TABLE 6. Sensitivity of CQ medium for the detection of total coliforms from treated and untreated water and the probability of obtaining a negative result when coliforms are present in the sample

| No. of coliforms CFU/100 ml[a] | | No. of samples | Negative ONPG reaction, % (P)[b] |
|---|---|---|---|
| Range | Mean | | |
| 1 | 1 | 10 | 20 ($1.35 \times 10^{-1}$) |
| 2–5 | 3 | 30 | 6.7 ($2.48 \times 10^{-3}$) |
| 6–10 | 7 | 5 | 40 ($8.32 \times 10^{-7}$) |
| >10 | 80 | 40 | 7.5 ($>3.26 \times 10^{-70}$) |
| CF | 1[c] | 75 | 2 ($1.35 \times 10^{-1}$) |

[a] Determined by enumeration of coliforms with the MF method. CF, confluent filters confirmed for total coliforms by positive reaction in BGB medium.
[b] Probability of obtaining the given value based on the number of positive and negative samples and the mean concentration of coliforms.
[c] Mean concentration was determined by assuming that at least one coliform had to be present.

produce the major portion of false-negative results, although the CL test produced a significantly higher number of false-negative results under these conditions than did the CQ test. Further, because filters with high background counts were placed into confirmatory media in the current study, as prescribed in the *Standard Methods* (1), a higher level of positive results may have been obtained with the MF method.

Work published by Hall and Moyer (12) and Covert et al. (4) indicated that a statistically significant difference existed between the multiple-tube fermentation test and the CL system for the detection of total coliforms. These findings showed that the multiple-tube fermentation test was superior in coliform detection. Their results differ from those reported by Edberg et al. on a national field survey, which indicated that the CL system gave better recovery of total coliforms (7). Our data now suggest that the MF method is superior to the CL system and equivalent to the CQ system if atypical colonies are taken into account as recommended in *Standard Methods* for the detection of total coliforms from treated and untreated drinking water supplies. The data in this study indicate the importance of including atypical colonies (dark red mucoid cells with or without nucleation) as recommended in *Standard Methods* (1) in obtaining accurate coliform occurrence data.

**Evaluation of field inoculation of samples.** As shown in Table 7, there was no statistically significant difference with the CL system between the data from samples inoculated in the field or in the laboratory. The CQ system was not tested in the field inoculation phase of the study. The data suggest that, if the sample collectors are properly trained, there

TABLE 7. Comparison of results with field and laboratory inoculation of CL samples with laboratory-inoculated total coliform MF results[a]

| Test results | No. of samples with the indicated result | | | |
|---|---|---|---|---|
| | CLF | CLL | MF | Total |
| Positive | 2 | 1 | 2 | 5 |
| Negative | 54 | 55 | 54 | 163 |
| Total | 56 | 56 | 56 | 168 |

[a] McNemar chi-square value, 0.42; $P > 0.05$. CLF, CL field inoculation; CLL, CL laboratory inoculation.

TABLE 8. Identification of organisms isolated from false-positive (F-P) and false-negative (F-N) samples

| Organism | No. of isolations[a] | | | |
|---|---|---|---|---|
| | CL ($n$ = 35) | | CQ ($n$ = 17) | |
| | F-P | F-N | F-P | F-N |
| *Citrobacter freundii* | 2[b] | 10 | 0 | 2 |
| *Escherichia coli* | 0 | 2 | 1[b] | 2 |
| *Enterobacter cloacae* | 0 | 2 | 0 | 1 |
| *Morganella morganii* | 0 | 2 | 0 | 0 |
| *Hafnia alvei* | 0 | 0 | 0 | 2 |
| *Klebsiella* spp. | 0 | 1 | 0 | 0 |
| *Citrobacter* spp. | 0 | 1 | 0 | 1 |
| *Aeromonas hydrophila* | 0 | 0 | 0 | 1 |
| *Enterobacter agglomerans* | 0 | 1 | 0 | 1 |
| *Klebsiella pneumoniae* | 0 | 1 | 0 | 0 |
| Centers for Disease Control enteric group 41 | 0 | 1 | 0 | 0 |
| *Enterobacter amnigenus* | 0 | 0 | 1[b] | 0 |
| *Klebsiella oxytoca* | 0 | 1 | 0 | 0 |
| Fluorescent *Pseudomonas* group | 0 | 1 | 0 | 0 |
| Unidentified | 0 | 5 | 2 | 2 |
| No isolations | 0 | 5 | 1 | 0 |
| Total | 2 | 33 | 5 | 12 |

[a] In certain instances more than one type of bacteria was isolated from a sample.
[b] These were not considered true false-positive results because the isolates were identified as coliforms.

appears to be no reason why the samples could not be inoculated in the field and carried on ice to the laboratory and incubated for the appropriate time period.

**Identification of bacteria from false-negative and -positive tests.** Of the 47 isolates identified during the study, only *Aeromonas* and *Pseudomonas* species would not be considered classic coliform organisms (Table 8). *Aeromonas hydrophila* and *Pseudomonas maltophila* have been shown to contain β-galactosidase but lack the permease to transport the substrate into the cell (8). The occurrence of *A. hydrophila* in water supplies is important, because it is a well-known opportunistic pathogen (14) and has been implicated as the causative agent of waterborne enteric infections (5). Both of these organisms have been suggested to produce possible interference. The results of this study indicate that this may be a possible explanation for false-negative results but would only explain a minor percentage of this type of result. Therefore, our findings generally agreed with the spiked experiments carried out by Edberg et al. (8) with a known strain of *A. hydrophila* at concentrations as high as $2 \times 10^4$ CFU/ml, in which no false-positive results occurred.

Identifications also showed that in approximately 75 and 67% of the false-negative samples for the CQ and CL tests, respectively, coliforms were present in the commercial preparation but did not produce ONPG hydrolysis after 24 h of incubation. These results indicate the importance of using field data and not water samples spiked with coliforms for establishing comparability of new methods and also the importance of having a verification step to validate the CL or CQ products when replacing an existing coliform method for a given water utility.

Interestingly, approximately 11% of the bacteria isolated were identified as *E. coli*. The presence of this organism has also been reported by Edberg et al. (6), who isolated *E. coli* with the MF technique in six instances and with the CL

medium only once. These data suggest that there may be a lack of substrate utilization by the *E. coli* inoculated into the CL medium. The inability of *E. coli* to use the MUG substrate may be due to injury (3), the inability of the substrate to enter the cell (13), lack of expression of the gene (10), or nonutilization of the MUG substrate by *E. coli* strains (2, 10).

The data presented in this paper suggest that the CQ product is superior to the CL product in the detection of coliforms. Further, using the CL system will result in a higher proportion of false-negative results than will using either the CQ system or M-endo-LES agar. High HPCs did not affect the occurrence of false-positive or false-negative results with the commercial preparations.

## ACKNOWLEDGMENTS

The research was supported by a grant from the Metropolitan Water District of Southern California.

We thank Richard McCleary for his invaluable help with the statistical analysis and the Hach Company for supplying the Coliquik medium at no cost.

## REFERENCES

1. **American Public Health Association.** 1986. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, D.C.
2. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of β-D-glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335–339.
3. **Clark, D. L., B. B. Milner, M. H. Stewart, R. L. Wolfe, and B. H. Olson.** 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**:1528–1534.
4. **Covert, T. C., L. C. Shadix, E. W. Rice, J. R. Haines, and R. W. Freyberg.** 1989. Evaluation of the autoanalysis Colilert test for detection and enumeration of total coliforms. Appl. Environ. Microbiol. **55**:2443–2447.
5. **Davis, W. A., J. G. Kane, and V. F. Garagus.** 1978. Human *Aeromonas* infections: a review of the literature and a case report of endocarditis. Medicine (Baltimore) **57**:267–277.
6. **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. Appl. Environ. Microbiol. **56**:366–369.
7. **Edberg, S. C., M. J. Allen, D. B. Smith, and the National Collaborative Study.** 1988. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple tube method. Appl. Environ. Microbiol. **54**:1595–1601.
8. **Edberg, S. C., and M. M. Edberg.** 1988. A defined substrate technology for the enumeration of microbial indicators of environmental pollution. Yale J. Biol. Med. **61**:389–399.
9. **Federal Register.** 1989. Drinking water; national primary drinking water regulations; total coliform: proposed rule. Fed. Regist. **54**:27544–27567.
10. **Feng, P., R. Lum, and G. W. Chang.** 1991. Identification of *uidA* gene sequences in β-D-glucuronidase-negative *Escherichia coli*. Appl. Environ. Microbiol. **57**:320–323.
11. **Geldreich, E. E., M. J. Allen, and R. H. Taylor.** 1978. Interferences to coliform detection in potable water supplies, p. 13–20. *In* C. W. Hendricks (ed.), Evaluation of the microbiology standards for drinking water. U.S. Environmental Protection Agency, Washington, D.C.
12. **Hall, N. H., and N. P. Moyer.** 1989. Evaluation of multiple tube fermentation test and the autoanalysis Colilert test for the enumeration of coliforms and *Escherichia coli* in private well water samples, p. 479–496. AWWA Technology Conference Proceedings, Philadelphia, Pa.
13. **Hartman, P.** 1990. Personal communication.
14. **Ketover, B. P., L. S. Young, and D. Armstrong.** 1973. Septicemia due to *Aeromonas hydrophila*: clinical and immunological aspects. J. Infect. Dis. **127**:284–290.
15. **Lewis, C. M., and J. L. Mak.** 1989. Comparison of membrane filtration and autoanalysis Colilert presence-absence techniques for analysis of total coliforms and *Escherichia coli* in drinking water samples. Appl. Environ. Microbiol. **55**:3091–3094.
16. **Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson.** 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. Am. Water Works Assoc. J. **73**:585–590.
17. **Otnes, G.** Personal communication.
18. **Reasoner, D. J., and E. E. Geldreich.** 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. **49**:1–7.
19. **Zar, J. H.** 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.