IDEXX
Literature Cover Sheet

IDEXX Library #: 6F

Topic: Colilert and Quanti-Tray in Wastewater/Recreation Water

Title: "Comparison of *Escherichia coli*, Total Coliform, and Fecal Coliform Populations as Indicators of Wastewater Treatment Efficiency"

Author(s): G. Keith Elmund, Martin J. Allen, Eugene W. Rice

Date: June, 1999

Source: Water Environment Research Volume 71, Number 3

Highlights:

- 453 wastewater samples were collected over the course of one year at two different waste water treatment plants (p.333)
- Samples were analyzed by m-FC (Standard Methods 9222E) and Colilert with Quanti-Tray and identified with API20E.
- "The Quanti-Tray technique...was an effective method for quantifying *E. coli*...in these waters."
- Colilert method "is direct, reliable, and easy to interpret."
- With Colilert, "quantitative results are available within 24 hours...This compares with 48 hours for temperature-tolerance confirmatory techniques."
- The authors agree with AWWA-1994, USEPA's Dufour-1977, Edberg-1988 and Rose-1996 that "use of *E. coli* rather than the traditional fecal coliform group to measure wastewater disinfection efficiency would provide greater public health protection benefits for users of recreational water and water supplies."

Comparison of *Escherichia coli*, Total Coliform, and Fecal Coliform Populations as Indicators of Wastewater Treatment Efficiency

G. Keith Elmund, Martin J. Allen, Eugene W. Rice

ABSTRACT: *Escherichia coli*, total coliform, and fecal coliform population data were collected from two wastewater treatment facilities, a subsurface flow artificial wetlands, and a receiving stream. Results are presented from individual wastewater treatment process streams, final effluent, and river sites upstream and downstream of the treatment facilities. The QuantiTray technique with 4-methylumbelliferyl-β-glucuronide-based Colilert media was an effective method for quantifying *E. coli* and total coliform populations in these waters. Thermotolerant *Klebsiella pneumoniae* present in the effluent from one treatment facility interfered with recovery of fecal coliforms on m-FC media using the delayed-incubation membrane filtration technique; *Klebsiella* interference was not observed in the enumeration of *E. coli* by the QuantiTray technique. Both stream standards and discharge permits can be revised to apply *E. coli* as the indicator of fecal contamination. The results support development of *E. coli*-based effluent and stream standards to protect public health. *Water Environ. Res.*, **71**, 332 (1999).

KEYWORDS: *Escherichia coli*, total coliform, fecal coliform, wastewater, disinfection.

Introduction

The U.S. Environmental Protection Agency (U.S. EPA) stated that *Escherichia coli*-based wastewater effluent and stream standards would best serve the public health (U.S. EPA, 1986). However, effluent and stream standards are currently based on fecal coliform measurements. To develop *E. coli*-based stream standards and corresponding National Pollution Discharge Elimination System (NPDES) permit limits, U.S. EPA and individual states would require numeric comparisons of treated wastewater effluent fecal coliform and *E. coli* data corresponding data from receiving streams.

Fecal coliform tests are intended to serve as quantitative indicators of extent of fecal contamination in water and wastewater (APHA, 1995). Criteria for an ideal microbial indicator of fecal contamination in water include the following: (1) it should be present in feces of humans and warm-blooded animals and occur in greater number than pathogens, (2) its potential for growth in the aquatic environment should be minimal and should never surpass those of pathogens, (3) it should be readily detectable by simple means and produce unique and characteristic reactions to provide unambiguous identification of the group, (4) it should always be present when pathogens are present, and (5) it should show increased resistance to disinfectants compared to pathogens (Allen and Edberg, 1995; Bonde, 1966; and McFeters et al., 1978). However, standard laboratory methods for measuring fecal and total coliforms do not meet the specificity and sensitivity of these five criteria. For example, fecal coliform methods typically enumerate *Klebsiella* spp., *Enterobacter* spp., and *Escherichia* spp. (Bagley and Seidler, 1977; Caplenas and Kanarek, 1984; and U.S. EPA, 1986). Similarly, the standard total coliform test can recover *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp., and *Escherichia* spp. (AWWA, 1994; Geldreich et al., 1978; and Seidler et al., 1981).

Traditional membrane filter (MF) and most probable number (MPN) tests for fecal coliform in wastewater are labor and materials intensive. Both tests require precise control of laboratory conditions and a high degree of technical skill to perform and interpret results. Because the traditional fecal (thermotolerant) method often overestimates true fecal number (i.e., the probability that pathogens survive through the treatment process), the wastewater operator may compensate for high coliform results by applying elevated levels of chlorine to ensure that NPDES permit limits are not exceeded. Such practices result in greater chemical costs and inadvertent production of chlorine-based disinfection byproducts that may also pose health risks (Rebhun et al., 1997).

Presence of *E. coli* is considered a specific indicator of fecal contamination and reflects the possible presence of enteric pathogens (APHA et al., 1995). The use of defined substrate 4-methylumbelliferyl-β-glucuronide- (MUG-) based monitoring methods to directly measure the presence/absence of *E. coli* and total coliforms in drinking water is well established in the literature (AWWA, 1994; Covert et al., 1992; Drinking Water, 1989; Edberg et al., 1988 and 1990; National Primary Drinking Water, 1991 and 1996; and Rice et al., 1990 and 1991). Corresponding studies on wastewater processes, treated effluent, and receiving streams have not been published.

Although originally designed to measure *E. coli* and total coliforms in drinking water, the QuantiTray–Colilert system (IDEXX Laboratories, Inc.) may be a method of choice to provide quantitative MPN *E. coli* data on treated wastewater effluent and quantitative data on extent of fecal contamination in receiving streams. Use of defined substrate MUG-based media to specifically detect *E. coli* is direct, reliable, and easy to interpret: *Escherichia coli* produces an enzyme able to cleave a fluorogenic substrate that is visible under UV light. The QuantiTray technique permits simultaneous enumeration of total coliforms and *E. coli* based on the MPN technique where a sealable bubble tray is substituted for test

tubes. After incubation, clear wells are negative for total coliforms, positive total coliform wells have a yellow pigmentation, and those wells that also fluoresce under UV light are positive for *E. coli*. The number of positive wells on each tray is counted and compared to a reference table that gives corresponding MPN count of total coliforms or *E. coli* per 100 mL. Quantitative results are available within 24 hours regarding extent of fecal contamination in water, wastewater, or a receiving stream. This compares to an average of 48 hours for temperature-tolerance confirmatory techniques.

The objective of this study was to gather quantitative background data on *E. coli*, total coliform, and fecal coliform populations in wastewater treatment processes, secondary treated wastewater effluent, and receiving stream. These data could be used for development of *E. coli*-based wastewater effluent and stream standards that better protect public health. In this study, data were developed on three different types of wastewater treatment processes and two different types of final effluent disinfection. *Escherichia coli*, total coliform, and fecal coliform data were also collected from receiving stream upstream and downstream from wastewater effluent discharge points.

Methodology

Wastewater Treatment Facilities. The City of Fort Collins, Colorado, operates two wastewater treatment plants: the Mulberry Water Reclamation Facility (MWRF) next to the Cache la Poudre River (CLPR) at river mile 42.75 from the South Platte River and the Drake Water Reclamation Facility (DWRF) at river mile 38.8. The MWRF is a 26 × 10^3 m^3/d (7 mgd) combined trickling filter–activated-sludge plant that uses UV for final disinfection; final effluent is discharged to CLPR. DWRF is a 91 × 10^3 m^3/d (24 mgd) activated-sludge plant that uses chlorine for disinfection with subsequent application of sulfur dioxide to quench remaining chlorine before discharge. The DWRF can discharge either to an irrigation ditch, a dedicated pipeline that provides power plant cooling water, or the river. In addition, a pilot-scale, subsurface flow artificial wetlands began operating in the fall of 1995 at DWRF to evaluate effectiveness of this process in polishing a portion of final effluent. Each cell of wetlands was operated at approximately 19 L/min (5 gpm) to yield a 2-day hydraulic detention time. Coliform data were collected on influent and effluent from the wetlands. Effluent from the wetlands was pumped back to the headworks of DWRF.

Sampling Locations. *Mulberry Water Reclamation Facility.* Samples for bacteriological analysis were collected monthly from the following locations in the treatment sequence: primary clarifier effluent, trickling filter effluent, intermediate clarifier effluent, and final clarifier effluent before and after UV disinfection. Final effluent samples after disinfection were tested daily (seven samples per week) for fecal coliforms and 5 days each week for *E. coli* and total coliforms. The study period was May 23, 1996, through February 28, 1997. Fecal coliform limits in the NPDES permit require effluent discharged from MWRF in any month not to exceed a moving 30-day geometric mean of 2350 organisms/100 mL or have any moving 7-day geometric mean maximum value exceed a standard of 4700 organisms/100 mL.

Drake Water Reclamation Facility. Coliform samples were collected monthly from the following locations in the treatment sequence: primary clarifier effluent, intermediate clarifier effluent, and final clarifier effluent before and after disinfection with chlorine. Final effluent samples were tested daily for fecal coliforms and 5 days each week for *E. coli* and total coliforms. The study period was February 20, 1996, through February 28, 1997. Fecal coliform limits in the NPDES permit for DWRF effluent depend on point of discharge. If discharge was to CLPR, the 7-day geometric mean maximum limit was 4480 organisms/100 mL and the 30-day geometric mean limit was 2240 organisms/100 mL. If discharge was to Fossil Creek Ditch, the 7-day maximum and 30-day limits were 4000 and 2000 organisms/100 mL, respectively. If discharge was to the Rawhide Power Plant, the 7-day maximum and 30-day limits were 12 000 and 6000 organisms/100 mL, respectively. For NPDES permit compliance calculations, "less than" fecal coliform counts were treated as the numeric value; for example, < 10 became 10.

Artificial Wetland Demonstration Project. A two-cell subsurface flow artificial wetland demonstration project began operation at DWRF in 1995. The primary purpose of the project was to evaluate effectiveness of an artificial wetland in converting nitrate to nitrogen gas and removing trace metals from treated wastewater. The artificial wetland consisted of parallel basins lined with a polyvinyl chloride membrane, filled with 20-mm-diam washed gravel, and planted with cattails. Hydraulic detention time in each basin was approximately 2 days. Influent to the wetland was the chlorine-disinfected–sulfur dioxide dechlorinated effluent from DWRF. Influent and effluent grab samples from the artificial wetland were tested approximately once each week from the period February 20, 1996, through February 28, 1997.

Cache la Poudre River. Samples were collected from CLPR approximately once each week during the study period. The following locations were sampled: the CLPR at the U.S. Geological Survey (USGS) Gage (06752260) located at Lincoln Street (river mile 43.80), at Mulberry Street (river mile 41.00), at the Nature Center (river mile 39.25), and at the USGS Boxelder Gage (06752280) located at river mile 37.90 downstream of DWRF. The Lincoln Street site is upstream of MWRF. DWRF did not discharge to the river during the course of the study.

Coliform Tests. Fecal coliforms were analyzed using m-FC agar in the delayed-incubation MF technique, method 9222E in *Standard Methods* (APHA et al., 1995). Total coliforms and *E. coli* were measured using the QuantiTray technique with Colilert media following manufacturer's instructions.

Verification and Identification of Organisms. Colonies from m-FC plates and organisms from QuantiTray wells were streaked for isolation on brain–heart infusion agar (BHIA) and MacConkey's agar. Oxidase negative organisms were further identified using API20E biochemical identification strips (bioMÉRIEUX VITEK, Inc., Hazelwood, Missouri).

Data Management and Calculations. Data were analyzed using SigmaStat (SPSS, Inc., Chicago, Illinois). The city's NPDES permit limits for fecal coliforms were based on two sets of calculations of daily results: a moving 7-day geometric mean of all daily values was calculated and maximum 7-day value reported. A moving 30-day geometric mean of all daily values was also reported monthly.

Results

Descriptive Statistics. There were 189 and 264 daily effluent data triplets (*E. coli*, fecal coliform, and total coliform) for the MWRF and DWRF, respectively. Figure 1 (MWRF) and Figure 2 (DWRF) depict $\log_{10}$-transformed counts/100 mL for each coliform group over the course of the study. The graphs show that effluent coliform data for both facilities varied not only in number

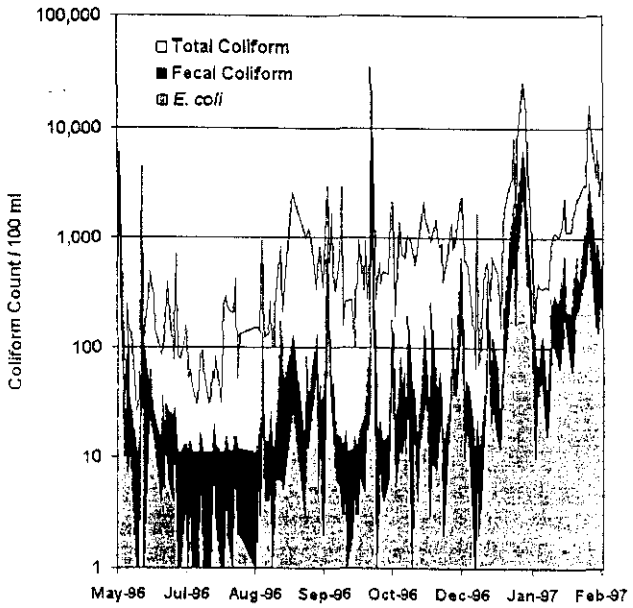

Figure 1—Comparison of daily *E. coli*, fecal coliform, and total coliform recoveries from MWRF effluent over time.

of coliforms recovered day-to-day but also in relative proportions of the three groups recovered each day. Variability in *E. coli* counts was similar to that observed for total and fecal coliforms. Over the course of the study, effluent *E. coli* counts ranged from 0 to approximately 10 000 organisms/100 mL at MWRF (n = 189) and from approximately 3 to 1500 organisms/100 mL at DWRF (n = 264). Effluent total coliform counts ranged from 0 to approximately 20 000 organisms/100 mL at both MWRF and DWRF. Effluent fecal coliform counts ranged from 0 to approximately

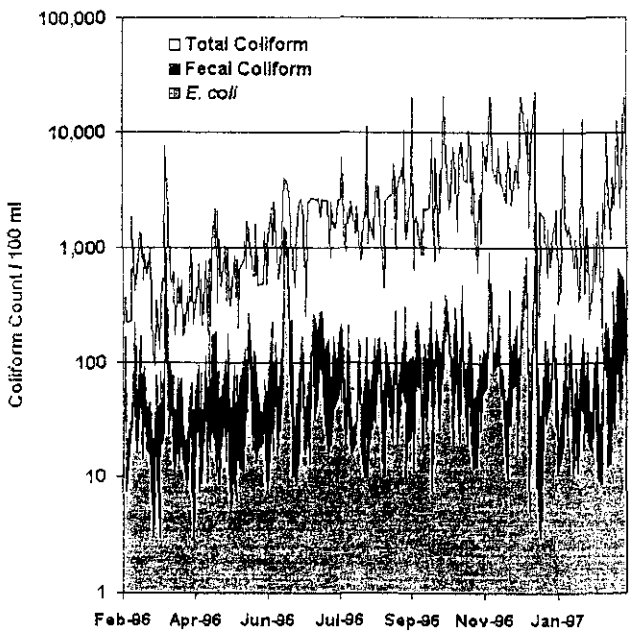

Figure 2—Comparison of daily *E. coli*, fecal coliform, and total coliform recoveries from DWRF effluent over time.

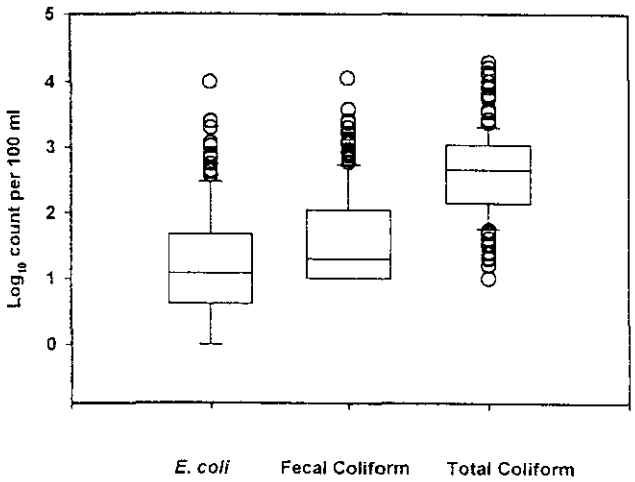

Figure 3—Box plot statistical comparison of *E. coli*, fecal coliform, and total coliform populations in MWRF effluent.

11 500 organisms/100 mL at MWRF and from 0 to approximately 1200 per 100 mL at the DWRF. Further evidence of day-to-day variability in the data was observed by comparing the mean and standard deviation of $\log_{10}$-transformed coliform counts. Mean fecal coliform counts at MWRF and DWRF were approximately 39 and 52 organisms/100 mL, respectively. Corresponding standard deviations about those means were approximately 5 and 3 organisms/100 mL, respectively. Mean *E. coli* counts at MWRF and DWRF were approximately 16 and 34 organisms/100 mL, respectively, and corresponding standard deviations about those means were 7 and 3 organisms/100 mL, respectively.

Box plots depicting percentiles and medians of $\log_{10}$-transformed effluent coliform data for MWRF (n = 189) and DWRF (n = 264) are presented in Figures 3 and 4, respectively. Ends of boxes depict the 25th and 75th percentiles with a line at the median, error bars define the 10th and 90th percentiles, and circles describe outlying points. Log_{10} transformation was chosen to

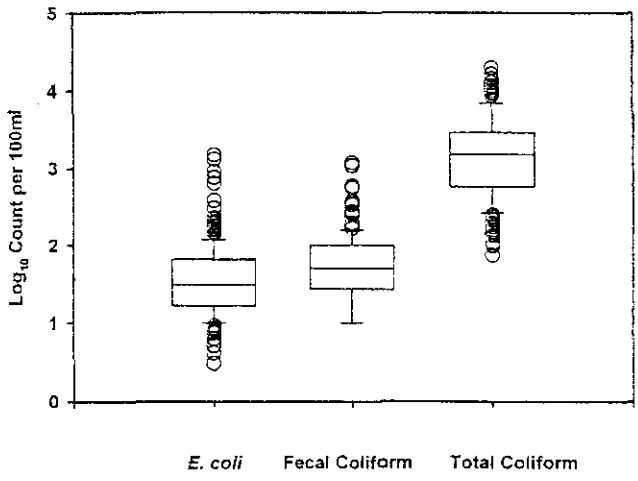

Figure 4—Box plot statistical comparison of *E. coli*, fecal coliform, and total coliform populations in DWRF effluent.

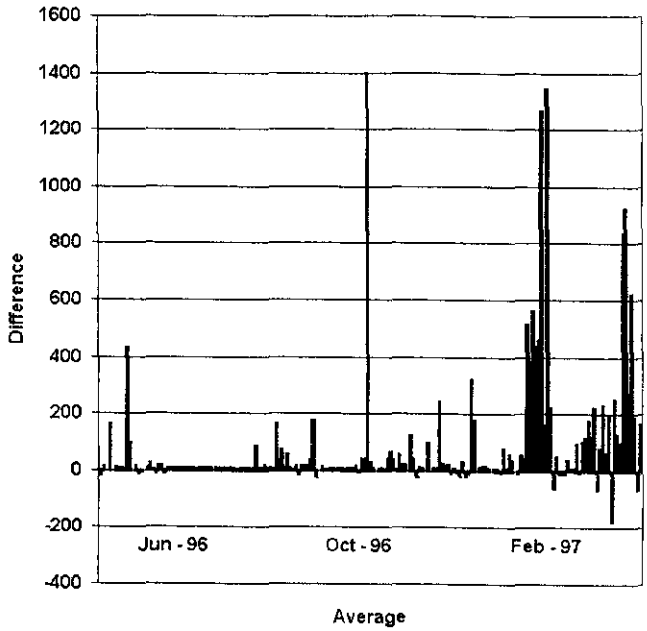

Figure 5—Average *E. coli* and fecal coliform counts versus difference between paired counts in the MWRF effluent over time.

facilitate visualization of descriptive statistics for sampled populations. Median counts of each coliform group in both effluents followed the general trend of *E. coli* < fecal coliform < total coliform. For all MWRF effluent values, median *E. coli*, fecal coliform, and total coliform counts were approximately 12, 20, and 460 organisms/100 mL, respectively. In contrast, corresponding median values for DWRF effluent were 31, 50, and 1500 organisms/100 mL, respectively.

Correlation Analyses. Arithmetic values of all effluent *E. coli*, fecal coliform, and total coliform counts from each plant were tested for normality. Normality was rejected for the three effluent coliform data sets (Kolmogorov–Smirnov [K–S] test, $P < 0.001$). For $\log_{10}$-transformed data, normality could not be rejected for effluent total coliform counts at MWRF ($n = 189$, K–S test, $P < 0.001$) and DWRF ($n = 264$, K–S test, $P < 0.001$). For arithmetic differences between paired counts ([total coliform − *E. coli*], [total coliform − fecal coliform], and [fecal coliform − *E. coli*]) normality was also rejected (K–S test, $P < 0.001$). For the difference between $\log_{10}$-transformed paired counts, normality (K–S test, $P < 0.001$) could not be rejected for 3 of the 12 combinations (MWRF [$n = 189$]: [$\log_{10}$ total coliform − $\log_{10}$ fecal coliform], [$\log_{10}$ total coliform − $\log_{10}$ *E. coli*], and DWRF [$n = 264$]: $\log_{10}$ fecal coliform − $\log_{10}$ *E. coli*). Because *E. coli*, fecal coliform, and total coliform data sets did not follow normal distributions, correlations between counts were evaluated using the Spearman rank order test. This nonparametric test measures the strength of association between pairs of variables without specifying which variable is dependent or independent and assumes that error distributions in the compared data sets are the same.

For the MWRF effluent ($n = 189$), the Spearman rank order correlation coefficient r_s comparing fecal coliform to *E. coli* counts was 0.809 ($P < 0.05$). Corresponding correlation coefficients comparing fecal coliform counts to total coliforms was 0.685 ($P < 0.05$) and *E. coli* counts to total coliform counts was 0.751 ($P < 0.050$). Correlation coefficients in this range suggest a moderate to high correlation (Sprinthall, 1982).

For the DWRF effluent ($n = 264$), the correlation coefficient r_s comparing fecal coliform to *E. coli* counts was 0.490 ($P < 0.05$). Although this value was lower than the correlation observed with MWRF data, it is considered moderate. Correlation coefficients comparing fecal coliform and *E. coli* counts to their paired total coliform data were 0.479 ($P < 0.050$) and 0.582 ($P < 0.05$), respectively. Correlation coefficients in this range are considered moderate (Sprinthall, 1982).

***Klebsiella* Interference with Fecal Coliform Measurements.** Figures 5 (MWRF) and 6 (DWRF) depict the average of daily *E. coli* and fecal coliform counts versus the difference between paired counts over time. The height of bar lines above 0 indicates the extent that a fecal coliform count exceeded its paired *E. coli* count; bar lines below 0 depict the opposite condition. For MWRF, marked differences between paired effluent fecal coliform and *E. coli* counts appear from October 1996 through February 1997. Comparable trends were not apparent in the DWRF effluent (Figure 6). Beginning in August 1996 and continuing through the early winter of 1997, technicians noted a predominance of two distinct colony morphologies on the m-FC agar plates. On m-FC medium, one set of colonies was uniform and blue with entire edges; these were typical *E. coli*. The other set of colonies was shiny with blue centers and entire edges. From this second set, technicians repeatedly recovered and identified thermotolerant *K. pneumoniae* (API 5215773 and 5205773) from MWRF effluent fecal coliform plates.

Mulberry Water Reclamation Facility Process Characteristics. Coliform counts from individual treatment processes at MWRF are shown in Table 1. Coliform counts declined as wastewater passed through the MWRF treatment processes. There was an approximate five-order of magnitude reduction in all tested populations (total coliform, fecal coliform, and *E. coli*) from primary clarifier effluent to final effluent after UV disinfection.

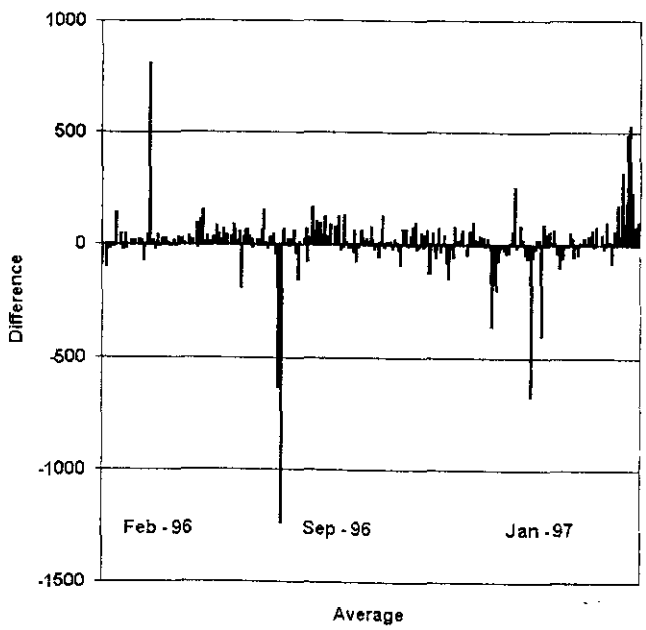

Figure 6—Average *E. coli* and fecal coliform counts versus difference between paired counts in the DWRF effluent over time.

Table 1—Average $\log_{10}$ coliform counts in treatment processes at MWRF.

Location	$\log_{10}$ fecal coliform/100 mL	$\log_{10}$ *E. coli*/ 100 mL	$\log_{10}$ total coliform/ 100 mL	*E. coli* to fecal coliform ratio	*E. coli* to total coliform ratio
Primary clarifier effluent, n = 19	6.29 s^a = 0.18	5.97 s = 0.32	7.00 s = 0.24	47% ± 1%	9% ± 1%
Trickling filter effluent, n = 19	5.61 s = 0.42	5.46 s = 0.36	6.40 s = 0.33	71% ± 1%	11% ± 1%
Intermediate clarifier effluent, n = 19	5.57 s = 0.61	5.35 s = 0.62	6.26 s = 0.63	59% ± 1%	12% ± 1%
Final clarifier effluent, n = 20	4.13 s = 0.80	3.86 s = 0.80	4.76 s = 0.89	54% ± 1%	13% ± 2%
Final effluent after UV disinfection	1.52 s = 0.68 (n = 281)	1.21 s = 0.85 (n = 189)	2.60 s = 0.66 (n = 189)	49% ± 5%	4% ± 3%

[a] s = sample standard deviation.

Because coliform counts in this study were derived using different and independent laboratory methodologies (MF for fecal coliforms and MPN QuantiTray for total coliforms and *E. coli*), caution is needed when comparing ratios of the three coliform groups within unit processes at the treatment plants. It is inferred from comparison of results of the two test methods that *E. coli* represent a substantial portion of coliform populations within unit processes (Table 1). In MWRF primary clarifier effluent, *E. coli* counts represented approximately 47% of the fecal coliform and 9% of the total coliform counts. In final effluent after UV disinfection, corresponding *E. Coli* values were 49 and 4%, respectively. In late August 1996 through the early winter months of 1997 (Figures 1 and 5), blooms of thermotolerant *K. pneumoniae* were detected in MWRF effluent fecal coliform samples tested on m-FC agar. Blooms were attributed, in part, to carbohydrate-rich wastewater from the numerous microbreweries in Fort Collins that discharged to MWRF and the seasonally higher wastewater temperatures (19 °C) observed during late summer and fall.

Drake Water Reclamation Facility Process Characteristics. Similar to results observed at MWRF, coliform populations at DWRF declined as wastewater passed through the treatment processes (Table 2), especially at final clarification. Populations of total coliforms, fecal coliforms, and *E. coli* at DWRF declined approximately five orders of magnitude from primary clarifiers to final effluent. It is inferred from the independent recovery methods, that the proportion of *E. coli* to fecal coliforms increased from approximately 58% (n = 35) in primary clarifier effluent to 74% (n = 264) in final effluent after disinfection with chlorine. It is also inferred that the proportion of *E. coli* to total coliforms dropped from approximately 16% (n = 35) in primary clarifier effluent to 3% (n = 264) in final effluent after disinfection with chlorine. During the study period, all coliform groups in the DWRF final effluent increased in numbers. This result paralleled increased levels of effluent total suspended solids that were not captured during final clarification. Final effluent fecal coliform levels complied with NPDES permit conditions (Figure 7). In contrast to MWRF, relative proportions of the coliform population groups in final effluent did not show seasonal changes (Figures 2 and 6) and interference from thermotolerant *Klebsiella* was not evident in the delayed-incubation fecal coliform test (Figure 6).

Artificial Wetland Demonstration Project. Coliform populations entering and leaving the wetland were consistently small (Table 2). Data from weekly counts (n = 32) showed that there was approximately a 50% reduction of *E. coli* and fecal coliform

Table 2—Average $\log_{10}$ coliform counts in treatment process streams at DWRF.

Location	$\log_{10}$ fecal coliform/100 mL	$\log_{10}$ *E. coli*/ 100 mL	$\log_{10}$ total coliform/ 100 mL	*E. coli* to fecal coliform ratio	*E. coli* to total coliform ratio
Primary clarifier effluent, n = 35	6.57 s^a = 0.18	6.33 s = 0.24	7.13 s = 0.36	58% ± 1%	16% ± 1%
Intermediate clarifier effluent, n = 35	6.28 s = 0.47	6.09 s = 0.37	7.02 s = 0.56	65% ± 2%	12% ± 2%
Final effluent after chlorine disinfection	1.66 s = 0.41 (n = 372)	1.53 s = 0.47 (n = 264)	3.14 s = 0.53 (n = 264)	74% ± 3%	3% ± 2%
Artificial wetlands influent, n = 32	1.67 s = 0.57	1.50 s = 0.51	2.90 s = 0.67	67% ± 5%	4% ± 3%
Artificial wetlands effluent, n = 32	1.18 s = 0.26	0.74 s = 0.57	2.69 s = 0.53	36% ± 7%	1% ± 2%

[a] s = sample standard deviation.

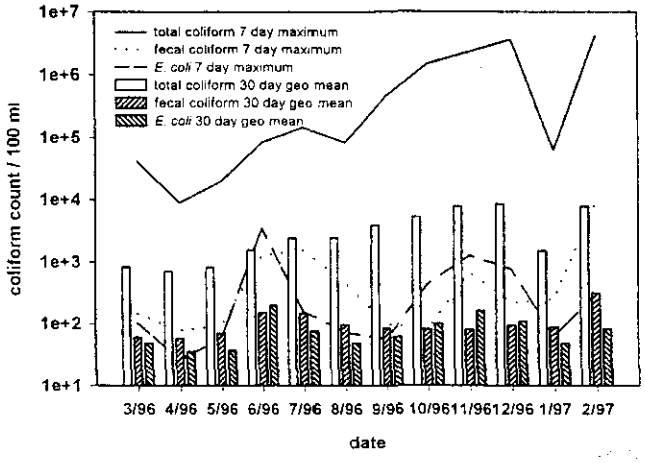

Figure 7—Moving 7-day maximum and 30-day geometric mean *E. coli*, fecal coliform, and total coliform counts in DWRF effluent over time.

counts as water passed through the wetland. However, there was only a slight reduction in total coliform counts. When comparing counts between the methods, *E. coli* represented 67% of fecal coliforms and 4% of total coliforms in the wetland influent. In wetland effluent, however, corresponding values dropped to 36 and 1%, respectively.

The Cache la Poudre River. Levels of all three coliform groups increased in river water as it passed through the community (Table 3). However, the increase was less than one order of magnitude in counts per 100 mL for all three groups over the 5.9 river miles studied. It should be noted that only MWRF discharged to the river during the course of this study. There was an approximate 68% increase in *E. coli* levels at the Mulberry Street site compared to the Lincoln Street site located upstream of MWRF. This increase was attributed to the MWRF effluent discharge. However, greater *E. coli*, total, and fecal coliform values observed at sites downstream from the Mulberry site were attributed to unmeasured and intermittent irrigation water return flows and storm water flowing to the river. Comparing the different recovery methods for all samples collected at each site shows that the apparent proportion of *E. coli* to fecal coliforms increased from approximately 41% at the Lincoln Street site (n = 38) to 93% at the Boxelder Gage (n = 39) located 9.5 km (5.9 mile) downstream. In contrast, apparent proportion of *E. coli* to total coliforms at the far upstream and downstream sites was relatively unchanged: approximately 14% (n = 38) and 11% (n = 39), respectively.

Discussion

The Fecal Coliform Test. Direct enumeration of enteric pathogens in water and wastewater is time consuming and ineffective and not a sensitive means to protect the public health (AWWA, 1994; Cherry et al., 1972; and WHO, 1993). Enteric pathogens such as *Salmonella, Shigella,* and *Vibrio* sp., if present in water or wastewater, appear in numbers too low for efficient recovery, growth, and identification in the laboratory (Allen et al., 1979; Cherry et al., 1972; and WHO, 1993). Moreover, there is no battery of laboratory tests that could detect every individual human pathogen.

Since before the turn of the century, it has been known that *E. coli* is possibly the best indicator of fecal contamination in water (Escherich, 1885) because it accounts for more than 95% of the coliform genera in human feces (Dufour, 1977, and Rice et al., 1990). Laboratory methods of that era did not provide a simple, reliable, and specific means to directly recover and quantify *E. coli*. However, in the early 1900s, laboratory culture methods for fecal coliform were developed based on the observation that most *E. coli* of fecal origin are thermotolerant. Fecal coliforms are the thermotolerant subset of total coliforms that grow at 44.5 ± 0.2 °C with gas production from lactose. Both MPN and MF techniques for detecting fecal coliforms are based on the observation that most fecal *E. coli* are thermotolerant. However, *E. coli* is not the only microbe able to grow at elevated temperature in laboratory media designed to recover and quantify fecal coliforms. Approximately 15% of *Klebsiella* are thermotolerant (Bagley and Seidler, 1977, and Caplenas and Kanarek, 1984) and approximately 10% of *E. coli* are not thermotolerant (Dufour, 1977, and Edberg et al., 1990). In this study, thermotolerant *Klebsiella* sp. interfered with accurate measurement of fecal coliforms by the delayed-incubation MF technique in effluent from MWRF.

During wastewater treatment, populations of *E. coli* and pathogens decline. This decline is usually attributed to both the inability of *E. coli* and pathogens to compete with other microorganisms in wastewater treatment processes and their inability to proliferate outside their host (warm-blooded animals) (Klock, 1971, and Rose et al., 1996). In contrast, three coliform genera (*Klebsiella, Enter-*

Table 3—Geometric mean coliform counts at locations on CLPR upstream and downstream of the water reclamation facilities.

Location	River mile from South Platte	Fecal coliform/ 100 mL	*E. coli*/ 100 mL	Total coliform/ 100 mL	*E. coli* to fecal coliform ratio	*E. coli* to total coliform ratio
Lincoln Street Gage, n = 38	43.8	42 s^a = 3	17 s = 4	122 s = 5	41% ± 4%	14% ± 3%
Mulberry Street, n = 39	41.0	54 s = 3	29 s = 3	270 s = 5	54% ± 3%	11% ± 2%
Nature center, n = 39	39.3	75 s = 3	40 s = 3	420 s = 4	54% ± 4%	10% ± 2%
Boxelder Gage, n = 39	37.9	78 s = 4	73 s = 4	680 s = 6	93% ± 4%	11% ± 2%

[a] s = sample standard deviation.

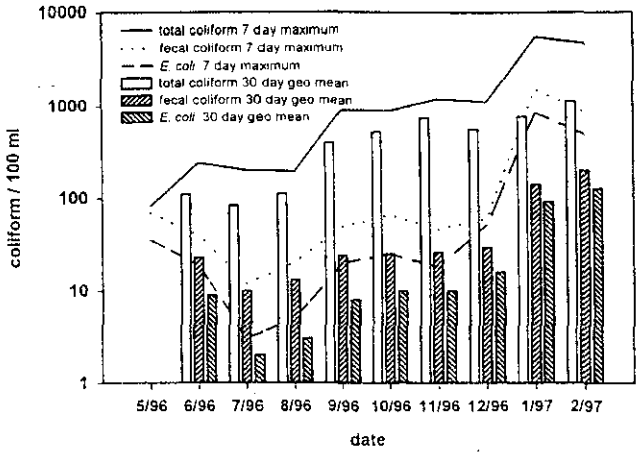

Figure 8—Moving 7-day maximum and 30-day geometric mean *E. coli*, fecal coliform, and total coliform counts in MWRF effluent over time.

obacter, and *Citrobacter*) that account for less than 3% of human fecal flora are able to proliferate in wastewater treatment processes (Niemi et al., 1995). Because some strains of *Klebsiella* are able to grow at 44.5 °C, they appear as fecal coliforms in standard MPN and MF fecal coliform tests. As was demonstrated with MWRF effluent, thermotolerant *Klebsiella* can be responsible for the erroneous detection and NPDES reporting of high effluent fecal coliform counts. Regulatory authorities, in turn, may make permit compliance and enforcement decisions based on possibly erroneous data regarding the extent of fecal pollution in wastewater discharged to receiving streams. Accordingly, a number of public health practitioners have advocated replacing the relatively nonspecific fecal coliform tests with direct MUG-based *E. coli* analyses (APHA, 1995; Drinking Water, 1989; National Primary Drinking Water, 1991 and 1996; Seidler et al., 1981; and U.S. EPA, 1986). The 1991 Total Coliform Rule for drinking water moved in this direction by allowing *E. coli* testing (National Primary Drinking Water, 1991), and the World Health Organization has abandoned traditional fecal coliform tests on drinking water entirely (WHO, 1993).

Meeting Projected Discharge Permit Limits. In this study, two wastewater treatment plants and an artificial wetland were able to easily meet their actual and projected NPDES permit limits based on fecal coliform and calculated *E. coli* standards at all discharge points. The most stringent limit for both treatment plants was discharge to CLPR. Possible discharge limits were calculated based on the assumption that new *E. coli* limits would be set at 67.5% of established NPDES fecal coliform limits. This percentage was derived from fecal coliform–*E. coli* standards developed for recreational waters (U.S. EPA, 1986). Recalculated limits may not accurately reflect site-specific discharge conditions for this or other water reclamation facilities but were used as an applied example. Under this assumption, the monthly 7-day maximum geometric mean limit for *E. coli* at MWRF would be 3200 organisms/100 mL and the 30-day geometric mean should not exceed 1500 organisms/100 mL. For DWRF, the corresponding 7- and 30-day *E. coli* limits would be 3000 and 1500/100 mL, respectively. For MWRF (Figure 8), the observed maximum 7-day geometric mean for *E. coli* was observed in January 1997 with a value of 853 organisms/100 mL and the highest 30-day geometric mean for *E. coli* was 127 organisms/100 mL in February 1997. The maximum 7-day geometric mean for *E. coli* at DWRF (Figure 7) was observed in June 1996 with a value of 198 organisms/100 mL. The highest 30-day geometric mean for *E. coli* was 61 organisms/100 mL observed in November 1996.

The ability to meet *E. coli*-based limits may not hold true for other wastewater treatment facilities with different NPDES permit discharge limits. If other wastewater facilities cannot meet permit limits based on *E. coli* standards, it would be apparent that optimization or upgrades of the treatment systems are warranted. Planning, financing, and construction of such upgrades will require sufficient time to reduce adverse effects on capital construction and operating budgets. Similarly, plans to upgrade wastewater treatment facilities to meet fecal-coliform-based NPDES permit limits may not be necessary if the facility can demonstrate treatment–disinfection effectiveness by directly measuring *E. coli* levels.

Conclusions

Based on these data and the research of others (AWWA, 1994; Dufour, 1977; Edberg et al., 1988; and Rose et al., 1996), use of *E. coli* rather than the traditional fecal coliform group to measure wastewater disinfection efficiency would provide greater public health protection benefits for users of recreational water and water supplies. Applying MUG-based technology to quantify *E. coli* would also simplify analytical procedures and reduce expenses associated with disinfection of effluent from wastewater treatment plants where thermotolerant *Klebsiella* and other nonfecal bacteria interfere with quantifying the extent of remaining fecal contamination. The World Health Organization has identified world trade and movement of agricultural produce as one of the greatest threats to public health in the future (Käferstein et al., 1997). Recent outbreaks of *Cyclospora* (CDC, 1996a, 1996b, and 1997a) and Hepatitis A (CDC, 1997b) associated with agricultural produce presumably contaminated with reused water make it imperative that public health officials move to the use of a specific and reliable indicator of fecal contamination: *E. coli*. The thermotolerant fecal coliform procedure developed in 1904 was useful to screen for *E. coli* when there were no other alternatives. However, it is now easy and relatively inexpensive to obtain quantitative data specifically and directly for *E. coli*. As this study and others have shown, nonfecal coliforms such as *Klebsiella* can actively increase during and after wastewater treatment and subsequently yield inaccurately high fecal coliform counts. Therefore, it is recommended that *E. coli* become the standard indicator for measuring wastewater disinfection efficacy. In addition to public benefits relating to microbial health threats, use of the more specific *E. coli* indicator may make it possible to reduce, or at least optimize, amount of disinfectant used to treat wastewater. In turn, this could reduce amount of neutralizer required to quench remaining chlorine. An additional benefit may be to reduce formation of disinfection byproducts subsequently discharged into receiving streams (Rebhun et al., 1997). Adoption of *E. coli*-based standards should also be considered for recreational water (streams, rivers, lakes, and reservoirs) as the fecal coliform method frequently overestimates true fecal levels in those waters.

Acknowledgments

Credits. This work was supported by the City of Fort Collins, Colorado. The authors gratefully acknowledge the Colilert supplies and use of the QuantiTray sealing apparatus provided by IDEXX Laboratories, Inc., and thank the management and staff of

the City's Pollution Control Laboratory, especially Cindy Wright-Jones, Ginger Wynne, and Forrest Schrupp, for their technical expertise in collecting samples, performing numerous bacteriological tests, and managing the data. The authors also thank Steven Edberg for his thoughtful and detailed critique of this manuscript. Disclaimer: the opinions expressed are those of the authors and do not necessarily represent the views of the City of Fort Collins, the AWWA Research Foundation, or the U.S. EPA.

Authors. G. Keith Elmund is the Environmental Services Manager, Utility Services, City of Fort Collins, Colorado; Martin J. Allen is Director, Technology Transfer, American Water Works Association Research Foundation, Denver, Colorado; and Eugene W. Rice is Microbiologist, Water Supply & Water Resources Division, National Risk Management Research Laboratory, U.S. EPA, Cincinnati, Ohio. Correspondence should be addressed to G. Keith Elmund, Environmental Services, 4316 LaPorte Ave., Fort Collins, CO 80521. Telephone: (970) 221-6691; Fax: (970) 416-2150; E-mail: kelmund@ci.fort-collins.co.us.

Submitted for publication November 20, 1997; revised manuscript submitted July 13, 1998; accepted for publication November 18, 1998.

The deadline to submit Discussions for this paper is September 15, 1999.

References

Allen, M.J., and Edberg, S.C. (1995) The Public Health Significance of Bacterial Indicators in Drinking Water. *Proc. Int. Conf.—Coliforms E. coli: Problem Solution.* R. Soc. Chem., Univ. Leeds, U.K., 176.

Allen, M.J.; Geldreich, E.E.; and Taylor, R.H. (1979) The Occurrence of Microorganisms in Water Main Encrustations. *Am. Water Works Assoc. Tech. Conf. Proc.*, Denver, Colo., 113.

American Public Health Association; American Water Works Association; and Water Environment Federation (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Ed., Washington, D.C.

American Water Works Association Research Foundation (1994) Comparison of the Colilert® Method and Standard Fecal Coliform Methods—90647. Denver, Colo.

Bagley, S.T., and Seidler, R.J. (1977) Significance of Fecal Coliform—Positive *Klebsiella. Appl. Environ. Microbiol.*, **33**, 1141.

Bonde, G.J. (1966) Bacteriological Methods for Estimation of Water Pollution. *Health Lab. Sci.*, **3**, 124.

Caplenas, N.R., and Kanarek, M.S. (1984) Thermotolerant Nonfecal Source of *Klebsiella Pneumoniae*: Validity of the Fecal Coliform Test in Recreational Waters. *Am J. Public Health*, **74**, 1273.

Centers for Disease Control (1996a) Outbreaks of *Cyclospora cayetanensis* Infection—United States, 1996. *Morbidity Mortality Wkly. Rep.*, **45**, 549.

Centers for Disease Control (1996b) Update: Outbreaks of *Cyclospora cayetanensis* Infection—United States and Canada, 1996. *Morbidity Mortality Wkly. Rep.*, **45**, 611.

Centers for Disease Control (1997a) Outbreaks of Pseudo-Infection with *Cyclospora* and *Crytposporidium*—Florida and New York City, 1995. *Morbidity Mortality Wkly. Rep.*, **46**, 354.

Centers for Disease Control (1997b) Hepatitis A Associated with Consumption of Frozen Strawberries—Michigan, March 1997. *Morbidity Mortality Wkly. Rep.*, **46**, 288.

Cherry, W.B.; Hanks, J.B.; Thomasson, B.M.; Murlin, A.M.; Biddle, J.W.; and Croom, J.W. (1972) Salmonella as an Index of Pollution of Surface Waters. *Appl. Microbiol.*, **24**, 334.

Covert, T.C.; Rice, E.W.; Johnson, S.A.; Berman, D.; Johnson, C.H.; and Mason, P.J. (1992) Comparing Defined-Substrate Coliform Tests for the Detection of *Escherichia coli* in Water. *J. Am. Water Works Assoc.*, **84**, 98.

Drinking Water: National Primary Drinking Water Regulations; Total Coliforms (Including Fecal Coliforms and *E. coli*); Final Rule. (1989) *Fed. Regist.*, **54**, 27544.

Dufour, A.P. (1977) *Escherichia coli*: The Fecal Coliform. In *Bacterial Indicators/Health Hazards Associated with Water.* A.W. Hoadley and B.J. Dutka (Eds.). Special Technical Publication 65, American Society of Testing and Materials, 48.

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.*, **56**, 366.

Edberg, S.C.; Allen, M.J.; Smith, D.B.; and 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 Fermentation Method. *Appl. Environ. Microbiol.*, **54**, 1595.

Escherich, T. (1885) Die Darmbakterien des Naugenborenen und Sauglings. *Fortschr. Med.* (Ger.), **3**, 515.

Geldreich, E.E.; Allen, M.J.; and Taylor, R.H. (1978) Interferences to Coliform Detection in Potable Water Supplies. In *Evaluation of the Microbiology Standards for Drinking Water.* EPA-570/9-78-00C, U.S. EPA, Washington, D.C., 13.

Käferstein, F.K.; Motarjemi, Y.; Bettcher, D.W. (1997) Foodborne Disease Control: A Transnational Challenge. *Emerg. Infect. Dis.*, **4**, 503.

Klock, J.W. (1971) Survival of Coliform Bacteria in Wastewater Treatment Lagoons. *J. Water Pollut. Control Fed.*, **43**, 2071.

McFeters, G.A.; Schillinger, J.E.; and Stuart, D.G. (1978) Alternative Indicators of Water Contamination and Some Physiological Characteristics of Heterotrophic Bacteria in Water. In *Evaluation of the Microbiology Standards for Drinking Water.* EPA-570/9-78-00C, U.S. EPA, Washington, D.C., 37.

National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria: Final Rule. (1991) *Fed. Regist.*, **56**, 636.

National Primary Drinking Water Regulations: Monitoring Requirements for Public Drinking Water Supplies; Final Rule. (1996) *Fed. Regist.*, **61**, 24351.

Niemi, R.M.; Niemelä, S.I.; Lahti, K.; and Niemi, J.S. (1995) Coliforms and *E. coli* in Finnish Surface Waters. *Proc. Int. Conf.—Coliforms E. coli: Problem Solution.* R. Soc. Chem., Univ. Leeds, U.K., 112.

Rebhun, M.; Heller-Grossman, L.; and Manka, J. (1997) Formation of Disinfection Byproducts During Chlorination of Secondary Effluent and Renovated Water. *Water Environ. Res.*, **69**, 1154.

Rice, E.W.; Allen, M.J.; and Edberg, S.C. (1990) Efficacy of β-Glucuronidase Assay for the Identification of *Escherichia coli* by the Defined-Substrate Technology. *Appl. Environ. Microbiol.*, **56**, 1203.

Rice, E.W.; Allen, M.J.; Brenner, D.J.; and Edberg, S.C. (1991) Assay for B-Glucuronidase in Species of the Genus *Escherichia* and Its Applications for Drinking Water Analysis. *Appl. Environ. Microbiol.*, **57**, 592.

Rose, J.B.; Dickson, L.J.; Farrah, S.R.; and Carnahan, R.P. (1996) Removal of Pathogenic and Indicator Microorganisms by a Full-Scale Water Reclamation Facility. *Water Res.* (G.B.), **30**, 2785.

Seidler, R.J.; Evans, T.M.; Kaufman, J.R.; Waarvick, C.E; and LeChevallier, M.W. (1981) Limitations of Standard Coliform Enumeration Techniques. *J. Am. Water Works Assoc.*, **73**, 538.

Sprinthall, R.C. (1982) *Basic Statistical Analysis.* Addison-Wesley Publishing Company, Inc., Reading, Mass.

U.S. Environmental Protection Agency (1986) Ambient Water Quality Criteria for Bacteria—1986. EPA-440/5-84-002, Cincinnati, Ohio.

World Health Organization (1993) *Guidelines for Drinking—Water Quality*, **1**, 95.