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Comparison of Membrane Filtration and Autoanalysis Colilert Presence-Absence Techniques for Analysis of Total Coliforms and *Escherichia coli* in Drinking Water Samples

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Over a 4-month period, 950 samples of treated drinking water were analyzed for total coliforms (TC) and *Escherichia coli* by both membrane filtration (MF) and Autoanalysis Colilert presence-absence (AC) techniques. The two tests agreed 97% of the time on the basis of presumptive TC results and 98.5% of the time on the basis of verified TC results. Samples which produced disagreement between the two tests were most often TC positive by MF and TC negative by AC. *E. coli* was recovered four times: twice by MF only, and twice by AC only but without the diagnostic fluorescence reaction. In two samples, *E. coli* could not be isolated from fluorescence-positive AC tests. On the basis of these results, the AC test was implemented as the routine analytical procedure for TC but not for *E. coli*.

The determination of total coliforms (TC) and fecal coliforms (FC) is the basis for evaluating microbiological quality of drinking water in Canada (7) and the United States (1). Procedures for TC analysis which are accepted by the regulatory agencies of these two countries include membrane filtration (MF), multiple-tube fermentation, and a presence-absence (PA) technique. FC may be enumerated using MF or multiple-tube fermentation techniques run parallel with or sequential to TC enumeration. When analyzed with these procedures, time-consuming subculturing and confirmation steps are required after the initial recovery of organisms. These confirmatory steps enable the differentiation of FC from TC. However, some TC which originate from nonfecal sources may be categorized as FC by traditional testing procedures (2). One way to overcome this weakness of traditional testing procedures is to analyze specifically for *Escherichia coli* rather than for FC.

Recently, the Autoanalysis Colilert (AC) test was introduced for the simultaneous determination of TC and *E. coli* (5). The AC test has several advantages over the traditional testing protocols, including its simplicity, specificity, and ease of inoculation and interpretation (5). The AC test is described by the manufacturer as enabling the unequivocal determination of *E. coli* after a maximum of 24 h of incubation time ("Colilert Product Information Portfolio," 1988). The AC test was shown to be equivalent to multiple-tube fermentation for TC analysis in a field evaluation which compared 46 samples from five sites by testing a large number of replicates from each sample (5).

The purpose of this study was to determine whether the AC test would yield equivalent results to traditional MF testing for TC and *E. coli* in distribution system water samples. It was decided to apply both MF and AC tests to samples from a large-scale distribution system monitoring program currently in place in Calgary. The program evaluates the microbiological quality of water in repaired water mains before they are returned to service and in new mains, replacement mains, and temporary service mains before they are put into service. This monitoring is designed to detect any microbial contamination which might have entered the water mains during repair work or during construction activities. Unacceptable heterotrophic plate count or TC results are cause for instituting flushing programs to restore water quality. This particular program is not one which is required to demonstrate compliance with regulations. Definitive, rapidly generated results are of paramount importance for repair work samples to minimize the exposure of consumers to water with elevated bacterial levels and to minimize the length of time that the mains are shut down. So, the AC test held promise as the analytical method of choice for this monitoring program. If the AC test could be shown equivalent or superior to MF testing for these types of samples, the usefulness of the AC test in microbiological emergency situations could also be evaluated (8).

Before implementing such a fundamental change in methodology, it was determined that AC and MF tests should be thoroughly examined. This report presents the results of a study to compare MF and AC on a large number of treated water samples collected over a 4-month period. The study differs from the original field evaluation (5) in that the number of samples and sampling sites in this project were large, and replicate samples were not analyzed.

MATERIALS AND METHODS

Samples were collected from building fixtures and fire hydrants after flushing procedures had been carried out according to *Standard Methods* (1) and City of Calgary *Standard Operating Procedures for Flushing Water Mains after Repairs* (6), respectively. Samples were collected into presterilized 250-ml plastic bottles (Systems Plus, New Hamburg, Ontario, Canada) which contained sodium thiosulfate (final concentration, 100 mg/liter) as a chlorine neutralizer. Samples were promptly placed in refrigerated coolers, transported to the laboratory, and analyzed within 4 h of collection.

Every sample was tested for heterotrophic plate count, TC by MF, and TC by AC. From the 250-ml plastic bottles, 1.0 ml of sample was withdrawn for heterotrophic plate count, 100 ml was withdrawn for the MF test, and the remaining volume was reduced to 100 ml for the AC test. The hetero-

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trophic plate count was enumerated by the MF technique with mHPC Agar (Difco Laboratories, Detroit, Mich.) as previously described (9). TC were enumerated by the single-step direct MF technique (Section 909A of reference 1) with mEndo agar LES (Difco) and membrane filters (0.45-μm pore size; Gelman, Ann Arbor, Mich.). Samples were classified as presumptively TC positive if metallic sheen colonies were observed. TC verification was performed by transferring growth from each metallic sheen colony to parallel tubes of lauryl tryptose Broth (LTB, Difco) and brilliant green bile broth 2% (BGB, Difco). An isolate was considered to be a verified TC if it grew in LTB with or without gas production and if it grew and produced gas in BGB. This is an accepted definition of TC typically used by water utilities (1). Species identifications were performed on all verified and nonverified sheen colony organisms with the API 20E identification kit (Analytab Products Ltd., St. Laurent, Quebec, Canada) after culture purification on Tryptic Soy Agar (Difco). The AC test was run with AC medium (Access Medical Systems, Branford, Conn.) in the PA format. The dehydrated medium was added to the 100 ml of sample remaining in the original plastic bottle and was incubated and interpreted as per the instructions of the manufacturer. Samples were classified as presumptively TC and *E. coli* positive on the basis of yellow color production and long-wave fluorescence, respectively. Loopfuls of all samples exhibiting positive reactions in AC were streaked on Tryptic Soy Agar within 2 h after samples were interpreted. Colonies with various morphology were selected and subjected to the same verification and identification procedures as were sheen colonies.

Quality control measures included laboratory instrument monitoring and culture medium checks. Blanks, duplicates, and positive and negative controls were used to validate every test run (1).

Statistical analysis. The data were analyzed by nonparametric statistical methods as specified by the U.S. Environmental Protection Agency for the analysis of the original Colilert equivalency testing data (5). These tests included the index of agreement, the kappa statistic (a chance-corrected index of agreement), and the z statistic.

RESULTS

Between May and August 1988, 950 samples were collected from 894 different sampling locations in the potable water distribution system. Most of these samples were collected from fire hydrants after connected water mains had undergone depressurization and repair, and the remainder were collected from fire hydrants connected to new or replacement water mains, from temporary services, and from plumbing fixtures in private residences. Of the 950 samples, 28 were repeat (check) samples collected from 28 previously sampled locations. For the purposes of this study, those 28 samples were considered independent of the original samples because (i) the repeat sampling was conducted at least 24 h after the original sample was collected and (ii) substantial water main flushing was performed before collecting the repeat sample. All samples were tested by both MF and AC.

Comparison of the MF and AC test results was complicated by two factors. First, the MF test yielded quantitative results and the AC test yielded qualitative results. This difficulty was overcome by treating the MF data as PA data, i.e., samples which had TC of ≥1/100 ml were considered as TC present, and samples which had TC of <1 were considered TC absent. Second, the classification of a sample as TC present or TC absent on the basis of sheen colonies (MF) or yellow color (AC) was in some cases not substantiated by the results of the verification testing. That is, not all sheen colonies confirmed as TC when subjected to verification testing, and not all AC samples exhibiting color changes could be shown to contain organisms which confirmed as TC. Thus, the results of the comparison differed depending on whether presumptive or verified TC data were used. The limitations of the traditional coliform verification procedure have been documented (10). For purposes of completeness, comparisons with both presumptive and verified data are presented.

TC recoveries from the distribution system water samples were very low by the MF test, at levels typical of City of Calgary treated water. Overall, the average number of metallic sheen colonies enumerated on the membrane filters was 0.15/100 ml, with a minimum of <1/100 ml and a maximum of 45/100 ml. Only 23 of the 950 samples produced metallic sheen colonies. In 14 of the samples, the sheen colony count was 1/100 ml of sample filtered. Of the 23 samples which produced sheen colonies, only 11 produced sheen colonies which could be verified as TC. Sheen colonies from the remaining 12 samples grew in both LTB and BGB, but gas was not produced in either medium.

TC recoveries were also in the same range by the AC test. Of the 950 samples, only 9 exhibited the yellow color change which indicated the presence of TC. Of these nine samples, seven yielded colonies which were verified as TC. As for the MF test, isolates from the two samples which were not verified as TC grew in both LTB and BGB but did not produce gas in either medium.

When presumptive sheen colonies on the MF test were compared with presumptive yellow color production in the AC test, the two tests gave the same result for 926 of the 950 samples (97%). Both tests indicated that 922 samples were TC negative and both tests indicated that 4 samples were TC positive. The tests gave different results for 24 samples. For 19 of these 24 samples, MF plates exhibited metallic sheen colonies but AC produced no color change. For 5 of the 24 samples, the AC test underwent a color change, but no metallic sheen colonies were observed on the MF plates.

On the basis of verified TC, MF and AC tests gave the same results for 936 of the 950 samples (98.5%). Both MF and AC categorized 934 samples as TC negative, and both tests categorized 2 samples as TC positive. The MF and AC tests gave conflicting results on 14 samples. MF identified nine samples as TC positive which the AC test had indicated were TC negative, while AC identified five samples as TC positive which MF had indicated were TC negative.

The differences between the MF and AC tests for TC recovery were not statistically significant whether presumptive or verified data were compared. Both procedures yielded false-positive (as defined by the traditional colony verification procedure) results. For the MF test, 12 of 23 samples (52%) were shown to be false-positives, i.e., they produced metallic sheen colonies which could not be verified as TC by the traditional fermentation tube tests. Of nine AC samples which produced a yellow color change, two (22%) were false-positives. When applied to the results of the verified TC for both tests (omitting the 922 samples initially identified as TC negative by both tests), the kappa statistic (kappa = 0.215) indicated a poor level of agreement between the two tests. However, the z statistic was large ($z = 6.812$), indicating that there was more agreement than chance alone would explain.

Both the MF and AC methods recovered isolates from the

TABLE 1. Species identifications (as performed by API 20E) and results of verification testing of organisms producing sheen colonies in MF tests and of organisms recovered from AC tests[a]

Organism	Sheen colony in MF test	Recovered in AC test
Citrobacter freundii	V, NV	V, NV
Enterobacter aerogenes	V	
Enterobacter agglomerans	V, NV	V, NV
Enterobacter amnigenus	V, NV	V
Enterobacter cloacae	V	V
Enterobacter intermedium	V	V
Escherichia adecarboxylata	NV	
Escherichia coli	V	V
Escherichia hermanii	V	
Escherichia vulneris	V	
Klebsiella oxytoca	V	V, NV
Klebsiella planticola	V	V
Kluyvera cryocrescens	NV	
Serratia liquefaciens	NV	

[a] V, Verified as TC; NV, not verified as TC; neither response indicates not recovered from that test.

four genera commonly accepted as belonging in the coliform group of organisms. *Enterobacter agglomerans* was most frequently recovered. However, *Enterobacter agglomerans* could only be verified as a TC once in five isolations; the criteria of growth in LTB and BGB plus gas production in BGB were not met. This organism was responsible for 33% of the false-positive results with the MF test. (Upon species identification, some nonverified sheen colonies were found to belong in the coliform group. However, these isolates were not considered TC in this study because they did not meet the traditional criteria.) Table 1 summarizes the genera and species recovered with each method and delineates the verification or nonverification of each species.

E. coli was recovered or indicated present in 6 of the 950 samples analyzed in this study. These recoveries are summarized in Table 2. For two of the six samples (samples A and B in Table 2), MF produced sheen colonies which were identified as *E. coli*; in the parallel test, AC did not produce fluorescence and *E. coli* were not identified. In two samples (samples C and D in Table 2), *E. coli* was recovered from AC tests which did not produce fluorescence; *E. coli* was not recovered from parallel MF tests. After identification as *E. coli*, both isolates were reinoculated into AC medium in pure culture. For the first isolate, fluorescence was observed after 36 h of incubation. However, the second *E. coli* isolate did not produce fluorescence upon reinoculation into AC medium.

DISCUSSION

Overall, the MF and AC tests were in agreement for TC analysis for 97% of the samples. Both tests gave TC negative results for 934 samples and both tests confirmed TC positive for two samples. The MF test was positive nine times (on the basis of verified sheen colonies) when the AC test was negative, and the AC test was positive five times (on the basis of verified TC) when the MF test was negative. However, this was not a statistically significant difference. This level of agreement is noteworthy, especially considering the very low levels of TC which were present in the samples. In such samples, the error associated with dividing the original sample into two subsamples for parallel testing is itself large (4).

Even though the disagreements between the two tests can be explained by sampling error, the differences which emerged between the two tests when individual samples were compared were disconcerting. The differences may be indicating that the tests were not measuring the same population. The large number of false-positive results from the MF test indicated a definite need to verify all sheen colonies,

TABLE 2. Details of *E. coli* recoveries from MF and AC tests

Sample	MF method (no. of sheen colonies)	API identity	AC method[a]		API identity
			Yellow color	Fluorescence	
A	22	*Escherichia coli*[b] *Escherichia hermanii* *Enterobacter agglomerans*	P	−	*Citrobacter freundii* *Escherichia hermanii* *Enterobacter cloacae*
B	1	*Escherichia coli*[c]	A	−	
C	3	*Escherichia adecarboxylata* *Enterobacter anmigenus* *Kluyvera cryocrescens*	P	−	*Escherichia coli*[d] Could not be identified
D	38 Heavy background growth	*Citrobacter freundii* *Enterobacter agglomerans* Could not be identified Could not be identified	P	−	*Escherichia coli*[b] *Serratia liquefaciens*
E	Confluent	Culture lost	P	+	*Citrobacter freundii* *Enterobacter cloacae* *Klebsiella oxytoca* *Klebsiella planticola*
F	0		P	+	Could not be identified Could not be identified

[a] P, Present; A, absent.
[b] Nonfluorogenic.
[c] Fluorogenic.
[d] Fluorogenic only after 24 h of additional incubation.

as is recommended by *Standard Methods* (1). The need to verify colonies is clearly a drawback when the rapid availability and correctness of TC results are important. The AC test is superior in this regard. With the AC test, classification of false-positives cannot be unequivocally proved. It is possible that a bona fide TC which caused a positive reaction may have been overgrown during incubation and may have been missed during the subculturing process.

With the low frequency of occurrence with which *E. coli* was recovered in these samples, differences in *E. coli* recovery between the two tests could not be defined. *E. coli* was recovered twice from MF plates but not from the corresponding AC tests, and it was recovered twice from AC tests but not from the corresponding MF tests. As was the case for the TC results, error associated with sample splitting could account for these differences. It was disturbing, however, that neither of the *E. coli*-positive AC tests exhibited fluorescence and that *E. coli* could not be isolated from either fluorescent AC test. For these samples, the AC test did not meet the specifications of the manufacturer for unequivocal determination of *E. coli*. It has recently been reported that 34% of *E. coli* from human fecal samples did not produce the fluorescence reaction (3); these nonfluorogenic *E. coli* could have been of this variety. Also, the identification of the organisms may be in doubt. These organisms were identified as *E. coli* by the API 20E system and by the Provincial Laboratory for the Public Health (Calgary, Alberta, Canada). However, they were identified as *Escherichia fergusoni* and *Escherichia vulneris* by the clinical microbiology laboratory at Yale University (New Haven, Conn.). So, either (i) nonfluorogenic *E. coli* yielded false-negatives by the AC test or (ii) some non-coli *Escherichia* species cannot be differentiated from *E. coli* by traditional identification protocols. Neither of these explanations is entirely satisfactory for a water utility monitoring water quality. Short of subculturing every TC-positive, fluorescence-negative AC test for *E. coli*, there is no way to know whether nonfluorogenic *E. coli* is being missed by the AC test.

The AC test has been implemented in our laboratory for analysis of distribution system samples for TC only. The AC test was superior to the MF test in the lower number of TC false-positives. Other advantages of the AC test included the ease of analysis and interpretation of results. At this time, the AC test is not used to assess samples for the presence or absence of *E. coli*. Species identifications are made on all organisms recovered from TC positive AC tests. Since species identifications would normally be performed on isolates from MF tests in our laboratory, there was neither gain nor loss in the area of *E. coli* identification as a result of implementing the AC test. A disadvantage of the AC test was the increased cost of materials over the MF test; this was, however, compensated by a corresponding decrease in labor costs with the AC test. AC would be employed in the event of a microbiological water quality emergency because of the correctness of TC results and the ability to substantially increase sample throughput in the laboratory.

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