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Evaluation of the Autoanalysis Colilert Test for Detection and Enumeration of Total Coliforms

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The Autoanalysis Colilert (AC) test was compared with the membrane filter (MF), 10-tube multiple-tube fermentation (MTF) technique, and the presence-absence test as described in *Standard Methods for the Examination of Water and Wastewater* for the detection and enumeration of total coliforms in water. The methods were evaluated with 31 samples from seven different sources. Each sample was analyzed by each of the techniques, using replicate 100-ml sample volumes. A total of 582 confirmed tubes were positive by the MTF test, and 533 tubes were positive by the AC test. Statistical analysis of the most-probable-number comparability data showed a statistically significant difference in the number of positive tubes, with the MTF test resulting in more positive tubes. There were no statistically significant differences in precision between the two methods. All the methods were comparable in detection of total coliforms. Levels of heterotrophic bacteria generally encountered in drinking water did not interfere with detection or enumeration of coliforms by the AC test.

The total coliform group of organisms is the principal indicator used to assess the microbiological quality of drinking water. The culture characteristics and the sanitary significance of coliform organisms have been studied extensively (3, 6, 12, 15, 16). The presence of any member of the coliform group in treated water suggests either inadequate treatment or contamination after disinfection. The coliform group is considered a reliable indicator of the adequacy of treatment and the potential presence of pathogens in the distribution system. The U.S. Environmental Protection Agency (USEPA) has specified two standard methods for the enumeration of total coliforms in drinking water (7, 8). These are the membrane filter (MF) method and the multiple-tube fermentation (MTF) method. Recently, the USEPA has proposed (9–11) the use of the presence-absence (P-A) coliform test (1) and the Autoanalysis Colilert (AC) test (4, 11) in addition to the previously approved MF and most-probable-number (MPN) tests.

The MF, MTF, and P-A coliform tests depend on the fermentation of lactose, producing sheen colonies, gas, or acid and gas. These methods require additional verification or confirmation of the presence of coliforms. The AC test was developed in response to these limitations and problems encountered in the New Haven, Conn., water distribution system with biofilm regrowth (4). The AC test is based on the ability of coliforms to produce the enzyme β-galactosidase, which hydrolyzes and cleaves the specific substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG), releasing *o*-nitrophenyl, which produces a yellow color. In addition, the enzyme β-glucuronidase produced by *Escherichia coli* forms a fluorescent substance when it hydrolyzes 4-methylumbelliferyl-β-D-glucuronide (MUG). This combination of substrates allows detection of both total coliforms and *E. coli* within 24 h.

The purpose of this study was to compare the AC test with the MF, MTF, and P-A coliform tests (1) for the detection and enumeration of total coliform bacteria.

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MATERIALS AND METHODS

Samples. A total of 31 samples were collected from seven different sources. Sample sources included natural springs, surface waters, well waters, cisterns, treated coagulated settled water from a drinking water treatment plant, and public drinking water supply distribution systems. Because of difficulties in locating public drinking water supply distribution samples with ambient levels of total coliforms, it was necessary to sample known coliform-positive sources in addition to public drinking water supply systems. Other reasons for sampling these sources were to evaluate the AC test with sample types having high heterotrophic plate counts (HPC) to determine whether there was coliform suppression and whether samples that have high mineral content and chromophores bias the AC test results. Samples were collected asceptically in 4-liter sterile polycarbonate sample bottles and returned to the laboratory and analyzed within 2 h of collection. Drinking water supply samples were collected in sterile sample bottles containing sodium thiosulfate (0.1 mg/liter). Initial MF total coliform analyses and HPC (1) with R2A medium (Difco Laboratories, Detroit, Mich.) were used to estimate the coliform densities and HPC prior to subsequent comparative analyses. Samples with total coliform levels of 20 or more coliforms per 100 ml were diluted in dechlorinated tap water to a theoretical level of 4 to 20 total coliforms per 100 ml to be representative of an unsatisfactory drinking water sample and to provide both positive and negative MPN tubes for evaluation purposes. Samples with fewer than 20 total coliforms per 100 ml were examined without dilution.

AC test. AC tubes (Access Medical Systems, Branford, Conn.) containing sufficient defined substrate for 10 ml of sample were prepared as a 10-tube MPN test. A P-A (AC) test was prepared by adding the contents of 10 tubes into a sterile screw-cap dilution bottle. Sample (10 ml) was added to each tube, and 100 ml of sample was added to each P-A (AC) bottle, and the powder was dissolved with agitation. The MPN AC tubes and P-A (AC) bottles were incubated at 35 ± 0.5°C for 24 h. Development of a yellow color indicated the presence of total coliforms. Doubtful positive tubes were

incubated for an additional 4 h and were also compared with a Colilert color comparator to assess any degree of yellow color. Each positive AC tube and P-A (AC) bottle was exposed to a hand-held long-wavelength (366-nm) UV light (Edmund Scientific Co., Barrington, N.J.). Fluorescence indicated the presence of *E. coli* (MUG test). A positive control (*E. coli*) was included with each sample.

MF test. Each sample was analyzed by the MF procedure (1) with 100-ml sample volumes. The GN-6 membrane filters (Gelman Sciences, Inc., Ann Arbor, Mich.) were placed in petri dishes (50 by 9 mm) containing 5 ml of either M-endo LES agar or M-endo agar (Difco Laboratories) and incubated for 24 h at 35 ± 0.5°C. The resulting sheen colonies were enumerated, and whenever possible, 10 sheen colonies were picked from each sample and inoculated into lauryl tryptose broth (LTB) and brilliant green-lactose-bile broth (BGLB) (Difco Laboratories) for verification as total coliforms (1, 2).

MTF test. The 10-tube MTF tests were performed by adding 10 ml of sample to each presumptive tube containing 20 ml of 1.5× LTB. The tubes were incubated at 35 ± 0.5°C, and positive presumptive tubes showing gas or heavy growth within 24 or 48 h were confirmed in BGLB (1).

P-A coliform test. P-A broth was prepared from a combination of lactose broth (Difco Laboratories), LTB, and bromcresol purple (Sigma Chemical Co., St. Louis, Mo.) according to *Standard Methods* (1). Sample volumes of 100 ml were inoculated into P-A culture bottles. The contents of the P-A bottles were mixed and incubated at 35 ± 0.5°C and examined after 24 and 48 h for acid and gas reactions. Positive P-A bottles were transferred into BGLB to confirm the presence of coliforms.

HPC. The HPC were performed according to *Standard Methods* (1) by the pour plate technique with R2A agar and plate count agar (Difco Laboratories). The plate count agar plates were incubated for 48 h and the R2A agar plates were incubated for 7 days at 35°C.

Test design. The following microbiological analyses were performed in triplicate on each sample: 10-tube AC test, 10-tube MTF total coliform test, MF total coliform test, and the P-A coliform test. A P-A (AC) test was performed on 15 of the 31 samples, and HPC were performed in triplicate on 16 of the 31 samples. All MPN estimates of coliform density were based on the number of confirmed tubes (1) or the number of positive tubes (yellow color) with the AC test. Whenever possible, five 24-h positive tubes, five 28-h positive tubes, and five 24-h negative AC tubes were chosen at random from each sample and streaked for isolation on MacConkey agar (Difco Laboratories). Isolates were identified by the API 20E system (Analytab Products, Plainview, N.Y.) (5).

Statistical analyses. The MPN recoveries (proportion of positive tubes) between the MTF (1) and the AC tests were compared by the Mantel-Haenszel test (17). The Mantel-Haenszel test analyzes a set of a 2 × 2 contingency tables by combining individual table results into a single test statistic. The contingency tables were developed by using the number of positive and negative tubes by each method as the columns and two methods as the rows. The proportions of positive tubes from the MTF (1) and AC tests were compared by determining whether the detection rate of positive tubes was the same for both methods by a chi-square statistic. The hypothesis tested was that there is no difference in detection rates by the two methods. Precision of the two methods was examined by comparing the variability among the number of positive tubes from the three replicate analyses. The sample variance was calculated from each method and sample and then compared by the Wilcoxon signed rank test (14). This test uses the sign and magnitude of the rank of the differences between the pairs of sample variances to determine statistical significance. All statistical tests were performed at the alpha = 0.05 level of significance.

Specificity tests. (i) AC ONPG test. A major question relating to the AC procedure centers on the detection of false-positive reactions. Organisms such as *Aeromonas* spp. which are not classically considered members of the total coliform group produce positive ONPG reactions with API 20E or other enzyme assays. Seven strains of *Aeromonas hydrophila* from clinical and environmental sources were spiked into dechlorinated tap water and analyzed by the AC test. All isolates were identified by the API 20E system and yielded positive ONPG results.

The cultures were grown in tryptic soy broth (Difco Laboratories) at 35°C for 18 h. Each culture was washed three times in phosphate buffer (1) diluted to the desired concentration, inoculated into dechlorinated tap water or filter-sterilized dechlorinated tap water, and analyzed by the AC test procedure. Filter sterilization was done with a prewashed filter (Durapore; 0.2-μm pore size; Millipore Corp., Bedford, Mass.) in a sterile glass filtration apparatus. When the organisms were spiked into nonsterile tap water, densities of *A. hydrophila* were estimated by membrane filtration with ampicillin-dextrin agar (13). In the filter-sterilized samples, the numbers of *A. hydrophila* were estimated by the spread plate procedure (2) with plate count agar. All samples were analyzed for the ability of the aeromonads to produce coliform-positive reactions with standard media used for enumerating coliforms. Samples were analyzed with LTB as an MTF test and M-endo LES agar with the MF technique.

(ii) AC MUG test. In the development of a MUG-containing medium for total gram-negative bacteria and *E. coli*, Petzel and Hartman (18) found that some *Flavobacterium* spp. were capable of producing β-glucuronidase, resulting in a positive MUG test. To test the specificity of the AC MUG test, cultures of *Flavobacterium* spp. from human, food, and environmental sources were spiked into sterile dechlorinated tap water and analyzed at various densities by the AC test procedure.

Flavobacterium cultures were grown in heart infusion broth (Difco Laboratories) at 35°C for 18 h. Each culture was washed three times in phosphate buffer and diluted to the desired density in filter-sterilized dechlorinated tap water. The numbers of *Flavobacterium* spp. at each dilution level were estimated by the spread plate technique (1, 2) with heart infusion agar and R2A agar, which were incubated at 35°C for 24 and 48 h, respectively. The *Flavobacterium* cultures were known to be MUG positive with the exception of the USEPA isolate, in which the MUG reaction was unknown.

RESULTS

Comparison of MTF and AC tests. A total of 582 tubes were positive (confirmed) by the MTF test (1), and 533 tubes were positive by the AC test. For the MTF analysis, the mean number of tubes positive per 30 inoculated per sample was 18.8, the median was 22, and the standard deviation was 11.4. For the AC test, the mean was 17.2, the median was 21, and the standard deviation was 11.8. Table 1 shows the number of positive tubes and samples analyzed by source.

TABLE 1. Comparison of *Standard Methods* (1) and AC MPN results

Sample source[a]	No. of positive tubes		No. of tubes inoculated
	Standard Methods	AC test	
Artesian well (4)	84	63	120
Spring (8)	199	195	240
Cistern (2)	26	42	60
Drinking water supply (2)	0	0	60
Settled water (6)	124	111	180
Surface water (4)	89	64	120
Well water (5)	60	58	150

[a] Number of samples analyzed.

Statistical analyses. (i) Recovery. The Mantel-Haenszel test applied to all 31 samples indicated a significant difference in the number of positive tubes between the two methods with the MTF test (1) resulting in more positive tubes ($\chi^2 = 12.5$, $P = 0.0004$). The MTF test (1) yielded more positive tubes than the AC test in 16 of the 31 samples, whereas the AC test yielded more positive tubes in only 5 of the samples. Both methods resulted in equal numbers of positive tubes in the remaining 10 samples (9 of 10 were either all positive or all negative).

(ii) Precision. No significant difference in method precision was detected ($P = 0.6966$). Replicate analyses varied by no more than two positive tubes in 94% of the samples analyzed by the MTF test (1) and 90% of the samples analyzed by the AC test. This also supports the conclusion of similar method precision.

Effect of HPC on suppression of total coliforms. The HPC ranged from 35 to 85,000/ml with R2A agar and from 60 to 9,000/ml with plate count agar. The HPC on all samples were higher with R2A agar than with plate count agar. Only one sample, a cistern sample, indicated the possibility of coliform suppression by high levels of heterotrophs. The replicate MF total coliform verified counts were 6, 7, and 6/100 ml, the MTF confirmed test values were 6.9, 6.9, and 12.0/100 ml, and the replicate AC test MPN values were all >23.0/100 ml. The mean HPC of the sample were 28,000/ml with R2A agar and 810/ml with plate count agar.

Comparison of coliform tests in detection of total coliforms. Of the 31 samples analyzed in this study, 26 (84%) were coliform positive by one or all of the methods. The total coliform levels ranged from <1 to 310/100 ml by the MF technique and from <1.1 to >23.0/100 ml by the MTF method. The percentage of positive presumptive tubes that were subsequently confirmed was 92%. The percentage of suspected coliform sheen colonies confirmed as coliforms by the MF technique was 82%. The percentage of positive P-A coliform tests that confirmed as total coliforms compared favorably (85%). The MTF and MF tests (1) detected 25 positive samples (81% of the total samples, 96% of all positives), whereas the P-A coliform test detected 26 positive samples (84% of the total samples, 100% of all positives). The AC MPN test compared favorably to the MTF and MF tests (1) in detecting 26 positive samples. The P-A (AC) test was also comparable in coliform detection to the MTF, MF, and P-A coliform tests. The P-A (AC) test did not detect coliforms in only 1 sample of 15 samples that were positive by the other methods. One sample was coliform negative by the MTF and MF procedures (1) but coliform positive by the AC MPN and P-A coliform tests.

Organisms isolated from AC test. Organisms isolated from the AC tubes were identified by the API system. Table 2 lists the organisms isolated along with the frequencies of isolation. *Enterobacter cloacae* was the most frequently isolated organism followed by *Enterobacter agglomerans*, *Klebsiella pneumoniae*, and *Serratia fonticola*. Of the 48 isolates from the 24-h positive tubes and 49 isolates from the 28-h positive tubes, 73 and 55%, respectively, were members of the total coliform group (*Enterobacter* spp., *Escherichia* spp., *Citrobacter* spp., and *Klebsiella* spp.). *E. agglomerans* was the most frequently isolated organism from the ONPG- and MUG-negative tubes. Each of the *Serratia* spp. isolates and the coliform isolates from the negative tubes were reinoculated into LTB and BGLB to confirm the ability to produce gas from lactose. Most (90%) of the *Serratia* spp. isolates produced gas in BGLB tubes, and five of nine coliform isolates (55.5%) produced gas in BGLB tubes. The remaining four coliform isolates were anaerogenic. Of the 533 ONPG-positive tubes, 4 were MUG positive. *E. coli* was isolated from three of these tubes, and *Salmonella* subgroup III was isolated from the fourth MUG-positive tube. *E. coli* was isolated from ONPG-positive tubes from two samples, but none of the AC tubes from those samples demonstrated fluorescence (MUG test).

TABLE 2. Organisms isolated from positive and negative AC tubes[a]

Isolate	No. of times isolated		
	24-h positive tubes	28-h positive tubes	Negative tubes[b]
Enterobacter cloacae	14	4	1
Enterobacter agglomerans	5	5	5
Enterobacter aerogenes	3	0	0
Enterobacter sakazaki	0	1	0
Escherichia coli	5	0	0
Citrobacter freundii	2	4	0
Klebsiella oxytoca	3	6	0
Klebsiella pneumoniae	3	7	3
Serratia fonticola	8	5	0
Serratia liquefaciens	1	7	1
Serratia marcescens	1	1	0
Serratia plymuthica	0	1	2
Serratia species	0	4	0
Aeromonas hydrophila	1	2	1
Hafnia alvei	0	0	1
Kluyvera species	0	1	0
Vibrio fluvialis	1	0	0
Pseudomonas maltophilia	0	0	1
Fluorescent *Pseudomonas* group	0	0	2
Shigella species	0	1	0
Salmonella subgroup III	1	0	0
Total no. of isolates[c]	48	49	17

[a] Identified according to the profile number determined by API.
[b] ONPG-negative and MUG-negative tubes.
[c] Does not include isolates not identified by API 20E.

AC ONPG and MUG specificity tests. Densities of *A. hydrophila* used to challenge the AC test ranged from 1.2×10^0 to 7.5×10^4 CFU/ml. Only one tube, at the high level of 7.5×10^4 CFU/ml, yielded a positive ONPG response. None of the isolates produced metallic sheen colonies on M-endo LES agar. All the isolates grew in LTB, producing turbid tubes, but only one environmental isolate produced gas in LTB after 48 h and not 24 h of incubation. However, the gas-positive tubes did not confirm in BGLB.

The results of the sterilized tap water samples spiked with

TABLE 3. AC results with dechlorinated, filter-sterilized tap water spiked with *Flavobacterium* spp.

Flavobacterium species	Source	CFU/ml[a]	AC reaction	
			ONPG	MUG
F. gleum	Human	1.2×10^9	−	−
		1.2×10^8	−	−
		1.2×10^7	−	−
F. multivorum	Human	6.8×10^8	+	−
		6.8×10^7	+	−
		6.8×10^6	−	−
F. thalophilum	Frozen vegetable	4.2×10^8	+	−
		4.2×10^7	+	−
		4.2×10^6	−	−
F. meningosepticum	Hamburger	2.8×10^8	+	−
		2.8×10^7	+	−
		2.8×10^6	−	−
F. multivorum	Creek water	4.3×10^8	+	−
		4.3×10^7	+	−
		4.3×10^6	+	−
		4.3×10^5	−	−
CDC group 11.b	Human	5.5×10^8	−	−
		5.5×10^7	−	−
		5.5×10^6	−	−
F. spiritivorum	Point-of-use water system	6.6×10^7	+	+
		6.6×10^6	−	+
		6.6×10^5	−	±[b]
		6.6×10^4	−	−

[a] CFU per milliliter in AC tubes with heart infusion agar and R2A agar.
[b] Very weak MUG reaction.

Flavobacterium spp. are presented in Table 3. *Flavobacterium* densities in the spiked samples ranged from a low of 6.6×10^4 CFU/ml with *Flavobacterium spiritivorum* to a high of 1.2×10^9 CFU/ml with *Flavobacterium pleum*. Both *Flavobacterium multivorum* isolates and three of the *Flavobacterium* spp. produced a positive ONPG reaction (yellow tube) at levels of 10^6 to 10^7 CFU/ml. Only one species, *F. spiritivorum*, produced a positive MUG test at the relatively high density of 6.6×10^5 CFU/ml (Table 3).

DISCUSSION

The AC test is a novel departure from classical total coliform cultural methods that depend on lactose fermentation to detect the presence of coliforms. The AC test uses the substrates ONPG (for total coliform) and MUG (for *E. coli*) both for essential nutrients and as the indicator system (yellow color and fluorescence). Organisms that are positive in classical ONPG or MUG tests generally will not be positive in the 24-h incubation time unless present in high numbers (4).

The AC test offers simplicity in that essentially all one does is add 10 ml of sample to the tubes or 100 ml of sample to a P-A bottle containing the defined substrate. The tubes or P-A bottles are incubated for 24 to 28 h at 35°C. Development of a yellow color indicates the presence of total coliforms. The test is designed so that no additional confirmation tests are needed. Incorporation of the two substrates (ONPG and MUG) allows a simultaneous MPN analysis or detection of total coliforms and *E. coli*. Since the AC test is provided in a ready-to-use powder form, there is no medium to prepare and sterilize. The AC tubes are reported to have an unrefrigerated shelf life of at least 1 year. Positive ONPG tubes are relatively easy to read, although with some samples containing low levels of stressed coliforms, the presence of yellow color is difficult to interpret. A positive MUG test is easy to detect, with the tubes or P-A bottles fluorescing brilliantly. The AC test was not affected by chromophores biasing the results, and samples high in mineral content did not interfere with the detection or enumeration of total coliforms.

Statistical analysis of data showed a significant difference in the number of positive tubes, with the 10-tube MTF test (1) providing more positive tubes than the 10-tube AC test. Although the difference was statistically significant, the number was small, only 49 tubes. No statistically significant differences in precision were found. Edberg et al. (4) reported in their national field evaluation of the AC test that more positive tubes resulted with the AC test (positive bias) compared with the MTF procedure (1). They also noted significant differences in precision, with the AC test being more precise than the MTF test (1) in two of five sites. The differences in precision and accuracy reported in these two studies may be due to differences in sample types; the national study used predominately public drinking water samples, whereas this study used predominately source water, cisterns, and well water diluted with dechlorinated tap water to simulate unsatisfactory drinking water samples. Another possible reason for the difference in the number of positive tubes is that the first 15 samples in the MPN comparability study were analyzed without the aid of the Colilert color comparator, which was not commercially available when the study was initiated.

There were no significant differences in the detection of total coliforms with the AC test used either as an MPN test or as a P-A test. There was only one sample in which the P-A (AC) test did not detect coliforms and the MTF, MF, and P-A coliform tests (1) did, and one sample in which the AC test detected total coliforms and the MF method failed to detect coliforms. Consistent with results reported by Edberg et al. (4), the detection or enumeration of total coliforms by the AC test was not affected by heterotrophic bacteria at levels of 500/ml or greater.

Although the AC test was comparable to the MTF test (1) in precision and accuracy and comparable to all the standard coliform tests in detection of total coliforms, the AC test does have limitations. As Edberg et al. (4) noted, the AC test is relatively refractory to high densities of heterotrophs; however, isolates of *A. hydrophila* and *Flavobacterium* spp. are capable of producing false-positive ONPG (yellow color) and MUG tests (Table 3). Petzel and Hartman (18) also reported *Flavobacterium* spp. isolates that yielded a positive MUG test. In addition, unpublished data from our laboratory have shown that extension of the incubation time beyond the 28-h period produced false-positive ONPG AC tubes from *A. hydrophila* and *Pseudomonas* spp. These data and the *Flavobacterium* and *Aeromonas* results reported in this study support the conclusion of Edberg et al. (4) that the AC test should not be applied to source waters, effluents, or samples other than drinking water supplies unless the analyst establishes the efficacy of the AC test with the particular sample type.

The identification of isolates from the AC test positive and negative tubes confirmed the specificity of the AC test. All genera of the total coliform group (*Enterobacter* spp., *Escherichia* spp., *Citrobacter* spp., and *Klebsiella* spp.) were recovered from positive AC tubes. Edberg et al. (4) found

that 82% of the isolates from positive AC tubes and 84% of the isolates from positive confirmed MTF tubes were members of the four genera classically considered in the total coliform group. In this study, 73% of the 24-h AC test positive-tube isolates, 55% of the 28-h positive isolates, and 23% of the isolates from the AC test negative tubes were members of the total coliform group.

Thirty-nine AC test negative tubes were selected for API identification. Seventeen of the tubes showed no growth when streaked on MacConkey agar, and isolates from five of the negative tubes could not be identified by the API identification system. The remaining 17 isolates from the AC negative tubes were identified by the API profile number (Table 2). Inclusion of the BGLB-positive *Serratia* spp. isolates and deletion of the anaerogenic coliform isolates from the tabulation of the AC test negative tubes based on the definition of a total coliform organism as referenced in *Standard Methods* (1) changed the percentage of coliform isolates. The percentage of total coliform isolates from 24-h positive tubes changed from 73 to 92%, and that from 28-h positive tubes changed from 55 to 84%. The percentage of organisms identified as total coliforms from negative tubes (false-negatives) changed from 23 to 20.5%. The percentage of *Serratia* spp. isolates reported in the national field evaluation from AC positive tubes was 12%. In this study, the percentage of isolates identified as *Serratia* spp. ranged from 7.7% in the AC test negative tubes to 20.8% in the AC test 24-h positive tubes. The differences in both the distribution and the identity of the microorganisms found in this study compared with results of the national field evaluation study can be attributed to the difference in sample types. Although the sample types differed in the comparison studies, the proportion or percentage of the isolates identified as members of the total coliform group from AC positive tubes was comparable whether one included or excluded the BGLB-positive *Serratia* spp. as total coliforms.

There were too few MUG-positive tubes and *E. coli*-positive samples to evaluate the efficacy of the AC test in detection of *E. coli*. *E. coli* was isolated from three of four MUG-positive tubes; however, *E. coli* was also isolated from two samples that were ONPG positive but did not exhibit a positive MUG response. These results suggest that not all isolates of *E. coli* are MUG positive by the AC test. More definitive studies are needed to evaluate the adequacy of the AC defined substrate test in detection of environmental isolates of *E. coli*.

In summary, the AC test can detect and enumerate total coliforms and *E. coli* from a water sample within 24 h with no additional confirmatory tests. The AC test is very easy to use, only requiring the addition of sample to the tubes, incubation for 24 h, and interpretation of the reactions. The AC test was comparable to the 10-tube MTF test (1) in detection and enumeration of total coliforms and comparable to the standard MF and P-A coliform tests in detection of total coliforms. Heterotrophic bacteria at levels usually reported in drinking water do not interfere with detection or enumeration of coliforms by the AC test.

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