APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 2004, p. 1242–1244 Vol. 70, No. 2
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.2.1242–1244.2004

Evaluation of Colilert-18 for Detection of Coliforms and *Eschericha coli* in Subtropical Freshwater

Kuo-Kuang Chao,[1] Chen-Ching Chao,[2] and Wei-Liang Chao[1]*

Department of Microbiology, Soochow University, Shih Lin, Taipei,[1] and Department of Soil Environmental Science, National Chung-Hsing University, Taichung,[2] Taiwan, Republic of China

Received 29 September 2003/Accepted 13 November 2003

The accuracy of Colilert-18 as a test for coliforms and *Escherichia coli* in subtropical freshwater was evaluated by using API 20E strips and fatty acid methyl ester analysis. The false-positive and -negative rates of detection were 7.4 and 3.5%, respectively, for *E. coli* and 9.6 and 6.3%, respectively, for coliforms.

In Taiwan, coliform bacteria are the recommended hygienic indicator organisms for both raw and potable water. However, many bacteria in the genera of *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia* meet the definitional criteria for coliforms even though they are not of fecal origin (8). Disagreements between government regulators and the water company sometimes relate to water that contains nonfecal coliforms, which interfere with the ability to accurately detect coliforms of fecal origin (the permissible limit in Taiwan being 20,000 CFU per 100 ml of raw water). As suggested previously, *Escherichia coli* could serve as a more specific indicator for fecal contamination of freshwater. The Republic of China Environmental Protection Agency has approved the use of two methods for the detection and enumeration of *E. coli* in water samples. One of them is a most-probable-number method based on the defined substrate technology using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-glucuronide (MUG) as the growth substrates (7).

A proprietary medium formulation and testing package incorporating these substrates has been marketed by IDEXX Laboratories (Westbrook, Maine) under the product name Colilert. The Colilert system yields results that are consistently similar to those of traditional standard detection methods for *E. coli* and total coliforms in freshwater (4, 5, 6). Colilert-18, a system intended to provide rapid (18-h) results, has been reported to detect coliforms and *E. coli* in potable water samples with an accuracy of 100% (10). However, other studies have disputed this degree of accuracy (14, 16). This dispute was of concern in our efforts to achieve rapid and accurate *E. coli* and coliform detection in our water monitoring program in Taiwan. Herein, we report the false-positive and false-negative rates of *E. coli* and coliform detection in subtropical freshwater samples obtained near metropolitan Taipei using Colilert-18.

Isolation of target organisms. Thirteen river water samples were collected from 11 different sites between June 2002 and January 2003. At each sampling date, following at least 3 consecutive days of clear weather, water samples were collected between 9 and 11 a.m. Water temperatures in the region averaged 28.5 ± 1.8°C (July-September) and 22.3 ± 0.9°C (October-December). Each sample was collected by immersing a 500-ml autoclaved polypropylene bottle in the middle of the river. The samples were placed on ice and transported back to the laboratory. The entire process, from sampling to the commencement of incubation, took less than 6 h. Detection of coliforms and *E. coli* by using the Colilert-18 (Quanti-Tray/2000) method was done according to the manufacturer's instructions. Yellow wells indicated coliform bacteria, and wells that were yellow and fluorescent when exposed to UV light (366 nm) indicated *E. coli*. Quanti-Trays showing less than 20 yellow wells (ONPG-positive) were selected for the recovery of coliforms and *E. coli*.

A total of 640 wells, consisting of 224 yellow and fluorescent wells, 359 yellow wells, 54 colorless wells, and 3 colorless and fluorescent wells, was selected for further study. Target organisms were recovered as described by Pisciotta et al. (14) by using sterile toothpicks to pierce the backing material of each Quanti-Tray. Liquid adhering to the toothpick was streaked onto m-ENDO (Difco, Detroit, Mich.) and m-TEC (Difco) agar plates for coliform and *E. coli* isolation, respectively. The m-ENDO plates were incubated at 35°C for 24 h. The m-TEC agar plates were incubated at 35 ± 0.5°C for 2 h and then transferred to 44.5 ± 0.2°C for 22 h. Organisms that formed yellowish (i.e., yellow, yellow-green, or yellow-brown) colonies on m-TEC agar and showed negative urease activity were classified as *E. coli*.

Accuracy for *E. coli* detection. When m-TEC agar was used to isolate *E. coli* from the ONPG-positive, MUG-positive (yellow and fluorescent) wells, 40 wells failed to yield colonies with typical *E. coli* characteristics (Table 1). Assuming that the false-negative rate for verified *E. coli* on m-TEC agar is less than 1% (3), the false-positive rate was 17.9% (40 out of 224) (Table 1).

Dufour et al. (3) reported that for freshwater samples, only 85% of the presumptive *E. coli* colonies that develop on m-TEC agar can be verified as *E. coli*. Furthermore, the inability of *E. coli* to form yellow colonies on m-TEC agar may simply be due to the absence of lactose permease (2). Fricker et al. (9) reported that approximately 10% of the coliforms isolated from surface freshwater lack this enzyme. Alternatively, some *E. coli* may simply fail to express β-galactosidase activity when

* Corresponding author. Mailing address: Department of Microbiology, Soochow University, Shih Lin, Taiwan, Republic of China. Phone: 886-2-28812523. Fax: 886-2-28831193. E-mail: wlchao@mail.scu.edu.tw.

TABLE 1. Verification of presumptive *E. coli* and nontarget Colilert-18 wells

Method used	No. and kinds of wells tested	*E. coli* identification		Percent that were:	
		Positive	Negative	FN[a]	FP[b]
m-TEC agar	224 YF[c] wells	184	40		17.9
	416 non-YF[d] wells	46	370	11.1	
FAME + API 20E	149 YF wells	138	11		7.4
	283 non-YF[e] wells	10[f]	273	3.5	

[a] FN, false negative.
[b] FP, false positive.
[c] YF, yellow-fluorescent.
[d] Includes 359 yellow wells, 3 colorless-fluorescent wells, and 54 colorless wells.
[e] Includes 240 yellow wells, 3 colorless-fluorescent wells, and 40 colorless wells.
[f] Includes 7 yellow wells and 3 colorless-fluorescent wells.

TABLE 2. Verification of presumptive coliforms and nontarget Colilert-18 wells

Method used	No. and kinds of wells tested	Coliform identification		Percent that were:	
		Positive	Negative	FN[a]	FP[b]
FAME analysis	240 yellow wells	217	23		9.6
	350 colorless wells	22	328	6.3	

[a] FN, false negative.
[b] FP, false positive.

incubated at 44.5°C (1). Therefore, further confirmation is needed.

To verify the above results, presumed *E. coli* isolates were isolated from 158 yellow and fluorescent wells by using m-TEC agar and were identified on the basis of their fatty acid methyl ester (FAME) profiles (12). *E. coli* ATCC 11775 (American Type Culture Collection, Manassas, Va.) was added to every tenth sample isolate to serve as a control. Of these presumed *E. coli* isolates, 56 could not be identified as *E. coli*. When these 56 isolates were reinoculated into the Colilert-18 medium, 9 isolates failed to develop *E. coli* characteristics. Hence, they were excluded from further analysis. The remaining 47 isolates were reidentified by using API 20E strips (bioMérieux, Inc., Hazelwood, Mo.) with *E. coli* ATCC 11775 as the control, and 36 isolates were positively identified as *E. coli*. Thus, in the present study, the use of Colilert-18 to survey subtropical freshwater produced a false-positive rate for *E. coli* detection of 7.4% (Table 1). This rate is considerably lower than the rate of 27.3% (3 out of 11 isolates) observed by Pisciotta et al. (14) when they used Colilert-18 and API 20E strips to assay for *E. coli* in Florida freshwater samples.

Although β-glucuronidase activity is used in the Colilert-18 system as a benchmark for *E. coli*, a sizable proportion (10 to 20%) of *E. coli* strains isolated from environmental sources are not MUG positive (17). Therefore, a false-negative evaluation is necessary. Suspensions from 416 wells, representing 359 yellow wells, 3 colorless-fluorescent wells, and 54 colorless wells, were plated onto m-TEC agar for isolation of *E. coli*. Of these 416 samples, 46 urease-negative, yellowish colonies were obtained, representing a false-negative rate for *E. coli* of 11.1%. Strain identification was also carried out to further confirm this result. Pure cultures were obtained from 283 Quanti-Tray wells, including 240 yellow wells, 3 colorless-fluorescent wells, and 40 colorless wells. Twenty-one isolates were identified as *E. coli* on the basis of their FAME profiles. These isolates were checked by growth in the Colilert-18 medium and subsequent API 20E reidentification. Only 10 isolates (7 from yellow wells and 3 from colorless-fluorescent wells) were actually *E. coli*. Therefore, the false-negative rate was approximately 3.5% (Table 1). A markedly higher false-negative rate of 11% has been reported for Colilert-18 by Schets et al. (16). In their study, they relied chiefly on the properties of lactose utilization and indole production at 44 ± 0.5°C as confirmation of *E. coli*. However, *E. coli* is not the only coliform with the ability to produce indole at this temperature (18). The present study's use of two direct identification systems may well produce a more accurate assessment of Colilert-18 than has hitherto been the case. If so, then the low false-negative rate shown in this study bodes well for the technique as a monitoring tool for subtropical freshwater.

Accuracy for coliform detection. For false-positive evaluation of coliforms, pure cultures were isolated from 240 yellow wells and identified based on their FAME profiles. Of the 23 isolates not identified as coliforms, 16 were *Erwinia carotovora*. Hence, the false-positive rate for coliform detection was about 9.6% (Table 2). On the other hand, of 32 isolates obtained from 350 colorless wells, 22 were coliforms, which makes the false-negative rate about 6.3%.

Previous studies that used membrane filtration methods (m-FC and m-TEC) to enumerate fecal coliforms and indole–methyl red–Voges-Proskauer–citrate tests and/or API 20E strips to confirm identification of fecal coliforms (13, 15) reported false-positive rates of 13 to 16% in temperate water and 30 to 36% in tropical water. The same studies reported false-negative rates of 1 to 2% in temperate water and 18 to 21% in tropical water. The present rates are markedly lower than the rates for tropical water. Another study (11) using the m-ENDO method to detect total coliforms in surface water, with subsequent API 20E identification, reported a 29.0% false-positive rate. Our observed 9.7% false-positive rate strongly supports our contention that the Colilert technology is a more accurate coliform detection system than membrane filtration methods.

It is clear that β-galactosidase exists in many gram-positive and gram-negative bacteria aside from *E. coli* (19). Hence, the Colilert-18 system tends to give higher total coliform counts than the traditional methods. In countries like Taiwan, where total coliform number still serves as the sole bioindicator for both treated and untreated water, conclusions based on Colilert data should be drawn with care. With this caveat, Colilert-18 is a satisfactory method for rapid screening for fecal contamination in subtropical freshwater.

This study was supported by the Environmental Protection Agency and the National Science Council, Republic of China (grants EPA-90-U1G1-02-101 and NSC90-2745-P-031-004, respectively).

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