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A New Membrane Filtration Medium for Simultaneous Detection and Enumeration of *Escherichia coli* and Total Coliforms

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Recovery of total coliforms and *Escherichia coli* on a new membrane filtration (MF) medium was evaluated with 25 water samples from seven states. Testing of the new medium, m-ColiBlue24 broth, was conducted according to a U.S. Environmental Protection Agency protocol. For comparison, this same protocol was used to measure recovery of total coliforms and *E. coli* with two standard MF media, m-Endo broth and mTEC broth. *E. coli* recovery on the new medium was also compared to recovery on nutrient agar supplemented with 4-methylumbelliferyl-β-D-glucuronide. Comparison of specificity, sensitivity, false positive error, undetected target error, and overall agreement indicated *E. coli* recovery on m-ColiBlue24 was superior to recovery on mTEC for all five parameters. Recovery of total coliforms on the new medium was comparable to recovery on m-Endo.

Diarrheal disease contracted from contaminated water continues to be a serious problem in developing countries and a lesser, but chronic, problem in developed countries (3, 18). Coliform monitoring is the preferred method for worldwide surveillance of drinking water quality (8, 13, 20, 33). Efforts are being made to improve techniques for coliform analysis, whether membrane filtration (MF), presence-absence (PA), or multiple tube fermentation, to obtain more information in less time.

One trend concerns an interest in MF media which allow determination of both total coliforms (TC) and *Escherichia coli* in a single incubation step. Seven such media have been described in the past 4 years (5, 7, 14, 15, 23, 24, 27). These media are designed as primary isolation media which allow distinction of TC and *E. coli* without transfer of filters or use of multiple incubation temperatures. Such media could accelerate compliance with U.S. Environmental Protection Agency (USEPA) regulations which require reexamination of drinking water samples found positive for total coliforms (13). Current USEPA rules mandate coliform analysis on a P-A basis. Consequently, MF procedures can be used in a P-A format and can also provide numerical data if desired. Numerical data are useful for the analysis of source water, recreational water, and wastewater, etc. Enumeration may also be required by international regulations.

The purpose of this study was to evaluate the newly developed TC-*E. coli* medium m-ColiBlue24 by using a P-A protocol mandated by the USEPA.

MATERIALS AND METHODS

Bacterial cultures. Coliform cultures used to determine the relative accuracy of m-ColiBlue24 were *E. coli* ATCC 25922, *Enterobacter cloacae* ATCC 23355, *Klebsiella pneumoniae* ATCC 13883, and *Citrobacter freundii* ATCC 8090. The first three were obtained from Bactrol Disks (Difco, Detroit, Mich.), and the fourth was obtained directly from the American Type Culture Collection. Working cultures were maintained in tryptic soy broth (Difco) plus 0.6% yeast extract plus 0.7% agar deeps.

Experimental protocols. Procedures for determination of false positive errors (FPE) and undetected target errors (UTE) were mandated in the specificity portion of reference 30. This document provides a protocol for the determination of the presence or absence of coliforms but not for their quantification. For each sample, FPE and UTE were determined simultaneously for recovery on m-ColiBlue24, m-Endo, and mTEC. Recovery of total coliforms on m-ColiBlue24 was compared to recovery of total coliforms on m-Endo. Recovery of *E. coli* on m-ColiBlue24 was compared to recovery of *E. coli* on mTEC and nutrient agar supplemented with 4-methylumbelliferyl-β-D-glucuronide (NA-MUG). Outlines of the procedures are as follows.

(i) TC FPE. Target colonies (red or partially red on m-ColiBlue24 and sheen or dark red on m-Endo) were picked from m-ColiBlue24 and m-Endo MF plates and struck onto Endo LES agar. All colonies picked from MF plates throughout this study were picked with sterile toothpicks under a dissecting microscope. After incubation for 20 to 28 h at 35°C, isolated colonies were struck onto nutrient agar plates. After incubation for an additional 20 to 28 h at 35°C, isolated colonies were inoculated into lauryl tryptose broth (LTB) tubes and incubated at 35°C for examination at 24 and 48 h. The same colonies were also tested for oxidase reaction as described below. Positive LTB tubes were used to inoculate brilliant green lactose bile (BGLB) tubes which were incubated at 35°C for examination at 24 and 48 h. Anaerogenic isolates were also identified to genus level by biochemical profiling with API20E strips (BioMerieux, St. Louis, Mo.).

(ii) TC UTE. Nontarget colonies (colorless on m-ColiBlue24 and pink or light red on m-Endo) were picked and struck onto nutrient agar. After incubation at 35°C for 20 to 28 h, isolated colonies were used to determine Gram stain and oxidase reactions. Gram-negative, oxidase-negative isolates were used to inoculate LTB tubes. These tubes were incubated at 35°C for examination at 24 and 48 h. Positive LTB tubes were used to inoculate BGLB tubes which were also incubated at 35°C for examination at 24 and 48 h. If any isolate yielded positive reactions in both LTB and BGLB it was reinoculated onto the original selective medium to determine whether it still failed to produce a typical target colony appearance. API20E analysis was also conducted on some isolates using colonies from the nutrient agar plates.

(iii) *E. coli* FPE. Target colonies (blue on m-ColiBlue24 and brown on mTEC) were picked and struck onto Endo LES agar. After incubation at 35°C for 20 to 28 h, lactose-positive colonies were struck onto nutrient agar which were incubated similarly. Isolated colonies from these plates were used for oxidase testing and API20E analysis.

(iv) *E. coli* UTE. Nontarget colonies (red on m-ColiBlue24 and nonbrown on mTEC) were picked and inoculated into EC broth and incubated in a 44.5°C water bath for 24 h. Positive tubes were used to streak nutrient agar plates which were incubated at 35°C for 24 h. Colonies from these plates were used to determine indole reactions. Isolates yielding a positive indole reaction were identified by API20E.

Calculations for FPE, UTE, sensitivity, and selectivity were based on the American Society for Testing and Materials Standard Practice D3870-91 (2) and the method described by Papasian and Hertlein (26).

Water samples. Twenty-five water samples, from seven states, were used to compare recovery of TC and *E. coli*. Out-of-state samples were shipped in Styrofoam coolers with Ice-Brix refrigerant. Analysis began within 24 h of the time samples were acquired at the sampling site (1). Local samples were returned to the laboratory for immediate analysis. Since m-ColiBlue24 is designed to recover both TC and *E. coli*, samples were used which contained both target populations. Nineteen samples were surface waters, three were nonchlorinated primary effluent from wastewater plants, and one was a potable water sample taken during a boil water alert. In addition, two spiked drinking water samples were prepared by adding wastewater to tap water to simulate contamination of potable water by fecally contaminated groundwater. In these two samples, the tap was flushed for approximately 10 min, water was collected aseptically, and chlorine levels were determined by the diethyl-*p*-phenylenediamine method with a DR3000 Spectrophotometer (Hach Company, Loveland, Colo.). After appro-

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TABLE 1. Comparison of *E. coli* recovery on two media

Medium	No. of reference isolates[a]		
	Positive	Negative	Total
m-ColiBlue24[b]			
Positive	234	6	240
Negative	0	250	250
Total	234	256	490
mTEC[c]			
Positive	198	32	230
Negative	5	235	240
Total	203	267	470

[a] Reference isolates were analyzed by a sequence of tests designated in an EPA protocol; see abbreviated description in Materials and Methods under "Experimental protocols."

[b] For m-ColiBlue24, 240 isolates were analyzed for FPE and 250 were analyzed for UTE. The results for all parameters were as follows: sensitivity, 100.0%; specificity, 97.7%; FPE, 2.5%; UTE, 0%; and overall agreement, 98.8%.

[c] For mTEC, 230 isolates were analyzed for FPE and 240 were analyzed for UTE. The results for all parameters were as follows: sensitivity, 97.5%; specificity, 88.0%; FPE, 13.9%; UTE, 2.5%; and overall agreement, 92.1%.

priate contact times, 1 ml of sterile 10% sodium thiosulfate solution was added per liter (1). Additional tests with chlorine-treated samples will be the subject of subsequent work. For all 25 samples, multiple replicates of several dilutions were filtered onto 0.45-μm-pore-size GN-Metricel membrane filters (Gelman, Ann Arbor, Mich.). Filters were placed on appropriate media as described below.

Media. m-ColiBlue24 was obtained from the Hach Company. Ampules of this filter-sterilized medium were used to saturate absorbant pads in Gelman 50-mm-diameter MF petri plates. Filters placed onto saturated pads were incubated at 35°C for 22 ± 2 h. On this medium, *E. coli* colonies are blue and all other coliforms are red. The composition of this medium is lactose, 0.6 g; casitone, 8.0 g; L-methionine, 0.1 g; yeast extract, 0.5 g; $MgSO_4$, 0.3 g; NaCl, 3.0 g; KH_2PO_4, 1.25 g; K_2HPO_4, 1.75 g; methylene blue, 0.016 g; triphenyl tetrazolium chloride, 0.07 g; sodium pyruvate, 1.0 g; erythromycin, 3.0 mg; Triton X-114, 0.5 g; 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid; cyclohexylammonium salt, 0.2 g; sodium azide, 0.02 g; and distilled water 1,000 ml (pH 7.0 ± 0.2). m-Endo broth MF (Difco) was used according to standard methods (1). mTEC medium (Difco) was used as previously described (32). For all three of these media, 1.8 to 2.0 ml of medium was used to saturate pads in petri dishes prior to addition of membrane filters. Although mTEC is typically used in an agar-solidified format, this modification was used to provide greater consistency with the m-ColiBlue24 and m-Endo data. Comparisons of recovery with agar solidification and saturated pads were performed with *E. coli* 11775, *E. coli* 11303, and *E. coli* 29194. Results showed that recovery on mTEC was not significantly different ($P < 0.05$) with the saturated pad format. Although FPE and UTE values were not determined for the NA-MUG method of Shadix et al. (28), this procedure was also used to estimate the number of *E. coli* colonies in some water samples. For experiments evaluating the relative accuracy of m-ColiBlue24, m-HPC agar (Difco) was used. Colonies were examined with a Unitron stereo binocular microscope at a magnification of ×10 (Unitron, Plainview, N.Y.). For determination of colony fluorescence during the NA-MUG experiments, a 6-watt ultraviolet lamp was used. Since the protocol used in this analysis was designed to evaluate media on a PA basis, all colonies picked were from plates with 1 to 5 colonies (31).

Relative accuracy of m-ColiBlue24. Coliform bacteria were grown overnight in tryptic soy broth plus 0.6% yeast extract and serially diluted in phosphate-buffered dilution water (1). Quintuplicate 1-ml quantities of appropriate dilutions were filtered and incubated on m-ColiBlue24. A heterotrophic plate count was similarly performed (1) with the same dilutions, and recovery data were used to determine relative accuracy.

Oxidase tests. Oxidase reactions were tested by standard methods (1). An alternative oxidase technique was also developed which allowed the rapid simultaneous testing of numerous colonies on a filter. Approximately 0.5 to 0.75 ml of oxidase reagent (Difco or BBL) was placed in the inverted lid of a standard 50-mm-diameter MF petri plate. A filter, with adhering 20- to 24-h colonies, was lifted into the lid so that the reagent soaked up through the filter. Within 15 to 20 s all oxidase-positive colonies develop the diagnostic blue color. This phenomenon is most readily observed under a dissecting microscope. This method is possible with m-ColiBlue24 because it is not subject to interference by medium acidification (16, 19).

Statistics. Tests comparing media for sensitivity, specificity, FPE, UTE, and overall agreement were carried out by standard statistical procedures for comparison of proportions in independent samples (29). For example, in comparing the level of sensitivity in m-ColiBlue24 with that in mTEC, a standard normal deviate was calculated by dividing the difference in observed sensitivities of the two media by the standard error of that difference. The result of the comparison was reported as the probability of a larger standard normal deviate assuming no difference in sensitivity in the two media.

For comparisons of enumeration, mean colony counts for the media were calculated for each of the multiple replicates of the 25 samples. Tests of no differences in colony counts among media were accomplished by using the single criterion of classification analysis of variance (29). The 25 samples were treated as separate studies. Results were interpreted by using treatment means and comparisons among treatment means, along with their standard errors.

RESULTS

Table 1 shows the sensitivity and specificity of *E. coli* recovery on m-ColiBlue24 and mTEC. Overall agreement with reference methods was 98.8% for m-ColiBlue24 and 92.1% for mTEC. Statistical analysis indicated that sensitivity, specificity, FPE, UTE, and overall agreement values for m-ColiBlue24 were all significantly different ($P < 0.05$) from those for mTEC.

Table 2 shows the sensitivity and specificity of TC recovery on m-ColiBlue24. The values in Table 2 were calculated by using three protocol modifications as follows: (i) a definition which recognized only isolates which produced gas in standard LTB and BGLB media as bone fide total coliforms (30), (ii) a protocol for identifying anaerogenic TC isolates by using additional API20E information, and (iii) a protocol for using the oxidase test as an ancillary test to screen typical TC colonies. Table 3 shows the specificity and selectivity of TC recovery on m-Endo. When anaerogenic TC were accounted for, (on the basis of API20E information) the TC FPE for m-ColiBlue24 and m-Endo were 26.8 and 29.0%, respectively. Statistical analysis of the sensitivity, specificity, FPE, UTE, and overall agreement data in Tables 2 and 3 obtained by use of the first protocol indicated that only the FPE values were significantly different ($P < 0.05$). Statistical comparison of the data in Tables 2 and 3 obtained by use of the second protocol indicated that none of the values for the five parameters were significantly different.

Table 4 compares the enumeration of *E. coli* in 25 samples recovered with three media. In three of four sample categories more *E. coli* were recovered on m-ColiBlue24 (i.e., the ratio

TABLE 2. TC recovery on m-ColiBlue24 by using three protocol modifications

Result by exptl method[a]	No. of reference isolates		
	Positive	Negative	Total
No correction			
Positive	120	130	250
Negative	2	248	250
Total	122	378	500
API20E			
Positive	183	67	250
Negative	2	248	250
Total	185	315	500
Ancillary oxidase			
Positive	206	44	250
Negative	2	248	250
Total	208	292	500

[a] No correction, TC identified without correction for anaerogenic isolates. Statistical results were as follows: sensitivity, 98.4%; specificity, 65.6%; FPE, 52.0%; UTE, 1.6%; and overall agreement, 73.6%. API20E, TC identified by using API20E to correct for anaerogenic isolates. Statistical results were as follows: sensitivity, 98.9%; specificity, 78.7%; FPE, 26.8%; UTE, 1.1%; and overall agreement, 86.2%. Ancillary oxidase, oxidase test used as an ancillary test to screen typical TC colonies. Statistical results were as follows: sensitivity, 99.0%; specificity, 84.9%; FPE, 17.6%; UTE, 0.96%; overall agreement, 90.8%.

TABLE 3. TC recovery on m-Endo by using two protocol modifications

Result by exptl method[a]	No. of reference isolates		
	Positive	Negative	Total
No correction			
Positive	141	109	250
Negative	5	245	250
Total	146	354	500
API20E			
Positive	149	61	210
Negative	5	245	250
Total	154	306	460

[a] No correction, TC were identified without correction for anaerogenic isolates. A total of 250 isolates were analyzed to determine both FPE and UTE. The statistical results were as follows: sensitivity, 96.6%; specificity, 69.2%; FPE, 43.6%; UTE, 3.4%; overall agreement, 77.2%. API20E, TC identified by using API20E to correct for anaerogenic isolates. A total of 210 isolates were analyzed to determine FPE and 250 isolates were analyzed to determine UTE. The statistical results were as follows: sensitivity, 96.8%; specificity, 80.1%; FPE, 29.0%; UTE, 3.2%; overall agreement, 85.7%.

between two media was >1.0), although the differences were significant for only four samples for the comparison with mTEC and for one sample for the comparison with NA-MUG. Table 5 indicates TC recovery on m-ColiBlue24 and m-Endo. The mean recovery ratio was >1.0 for two of four sample categories, but the number of samples for which the differences were statistically significant ($P < 0.05$) was small.

Table 6 lists the identities of isolates that did not produce gas in LTB or BGLB tubes and were therefore initially counted as noncoliforms per protocol but were found to be anaerogenic TC. Table 7 is a compilation of target TC colonies on m-ColiBlue24 and m-Endo which were false positives. The majority of false positives on both media were *Serratia* spp., *Aeromonas* spp., or *Vibrio fluvialis*. The 10 *Staphylococcus* sp. isolates on m-ColiBlue24 all came from a single sample. No other gram-positive isolates were found throughout the study.

The API20E identification codes of *E. coli* isolates recovered on m-ColiBlue24 and mTEC were compared. The same codes appeared to predominate with both m-ColiBlue24 and mTEC since four profile codes accounted for 71.7% of m-ColiBlue24 isolates and 79.3% of mTEC isolates. Excellent or very good identification codes were obtained for 96.5% of m-ColiBlue24 isolates and 95.9% of mTEC isolates.

Relative accuracy of the new medium was determined by comparing the recovery of four laboratory-grown coliform cultures on m-ColiBlue24 and on the nonselective medium m-HPC. m-ColiBlue24 recovered 93.2% of the laboratory-grown *E. coli* recovered on m-HPC. Of the three TC tested, m-ColiBlue24 recovered 94.8% of the *C. freundii*, 104.5% of the *Enterobacter cloacae*, and 10.6% of the *K. pneumoniae* isolates recovered on m-HPC. Of these four comparisons, only the last difference was statistically significant. Since TC from m-ColiBlue24 and m-Endo which were LTB and BGLB positive were not identified, comparison of overall *Klebsiella* spp. recovery on m-ColiBlue24 and m-Endo could not be made. However, the recoveries of anaerogenic *Klebsiella* spp. on these two media were similar, with eight isolates recovered on m-ColiBlue24 and seven isolate recovered on m-Endo (Table 7).

TABLE 4. Quantitative recovery of *E. coli* from 25 samples with three media

Sample category	Mean recovery ratio	
	m-ColiBlue24/ mTEC (CFU)	m-ColiBlue24/ NA-MUG (CFU)
Surface water ($n = 19$)	4.05[a]	2.00[b]
Sewage ($n = 3$)	3.29	2.28
Tap water spiked with sewage ($n = 2$)	1.09	0.72
Tap water during boil water alert ($n = 1$)	ND[c]	1.00

[a] Quantitative recovery of *E. coli* on m-ColiBlue24 was significantly greater ($P < 0.05$) than on mTEC for four samples. Quantitative recovery on mTEC was significantly greater than on m-ColiBlue24 for one sample.
[b] Quantitative recovery of *E. coli* on NA-MUG was significantly greater than on m-ColiBlue24 for one sample.
[c] ND, not determined.

TABLE 5. Quantitative recovery of TC from 25 samples with two media

Sample category	m-ColiBlue24/ m-Endo mean recovery ratio (CFU)	No. of samples with significant differences in recovery of TC ($P < 0.05$)
Surface water ($n = 19$)	3.94	3[a]
Sewage ($n = 3$)	1.37	0
Tap water spiked with sewage ($n = 2$)	0.59	1[b]
Tap water during boil water alert ($n = 1$)	0.85	0

[a] The number of TC recovered on mColiBlue24 was significantly greater than that on m-Endo for two samples; the number of TC recovered on m-Endo was significantly greater than that on m-ColiBlue24 for one sample.
[b] The number of TC recovered on m-Endo was significantly greater than that on m-ColiBlue24 for one sample.

DISCUSSION

m-ColiBlue24 compared favorably to existing media for TC and *E. coli* recovery from a geographically diverse set of samples. Recovery of *E. coli* on m-ColiBlue24 was better than that on mTEC as measured by sensitivity, specificity, FPE, UTE, and overall agreement. Though the EPA protocol used was not intended to provide quantitative evaluation of new media, a preliminary comparison of *E. coli* recovery on three media was possible. This revealed that m-ColiBlue24 recovered more *E. coli* than the mTEC or m-Endo–NA-MUG medium, al-

TABLE 6. Anaerogenic TC isolates

Bacterium	No. of isolates recovered on:	
	m-ColiBlue24	m-Endo
Klebsiella oxytoca	4	2
K. pneumoniae	4	2
Klebsiella sp.	0	3
Enterobacter cloacae	15	1
Enterobacter agglomerans	18	13
Enterobacter sakazaki	1	0
Enterobacter taylorae	2	0
Enterobacter aerogenes	1	0
Enterobacter intermedia	0	2
Enterobacter sp.	4	0
C. freundii	10	1
Citrobacter sp.	1	0
E. coli	3	0
Total[a]	63/250	24/210

[a] Identification of anaerogenic TC isolates was determined for 250 isolates on m-ColiBlue24 and 210 isolates on m-Endo.

TABLE 7. Identification of false positive isolates recovered on m-ColiBlue24 and m-Endo

Bacterium(-a)	No. of isolates recovered on:	
	m-ColiBlue24	m-Endo
Serratia spp.	17	11
H. alvei	9	1
Y. enterocolitica	2	0
Aeromonas spp. or *V. fluvialis*	21	39
Leclercia adecarboxylata	3	1
Ewingella americana	1	0
Staphylococcus spp.	10	0
Morganella morganii	0	1
No code or no growth	4	8
Total (%)[a]	67/250 (26.8)	61/210 (29.0)

[a] Identification of false positive isolates was determined for 250 isolates on m-ColiBlue24 and 210 isolates on m-Endo.

though only a few individual differences were statistically significant. *E. coli* colonies were easier to detect on m-ColiBlue24 because it was not necessary to transfer filters either from one medium to another or between incubators or to use a UV lamp. API20E codes obtained when isolates were tested indicated that the distributions of *E. coli* strains recovered were similar on m-ColiBlue24 and mTEC. The percentages of indole-negative *E. coli* isolates recovered were also similar (3.3% on m-ColiBlue24 and 3.0% on mTEC).

Brenner et al. (5) reported similar values after analyzing *E. coli* recovery on their new TC-*E. coli* medium. Specificity for *E. coli* target colonies was 95.7% and the FPE value was 4.3%. Brenner et al. reported that *E. coli* target colonies were clearly visible on their medium when TC colonies were too numerous to count. This was also true with m-ColiBlue24. Orenga et al. (24) reported that 5 of 94 *E. coli* strains were colorless or did not grow on their new TC-*E. coli* medium, resulting in a UTE value of 5.3%. They also found that 2 of 60 non-*E. coli* coliforms gave a typical *E. coli* reaction resulting in an FPE value of 3.3%.

Recovery of TC on m-ColiBlue24 was compared to that on m-Endo. The UTE value was low for both, 3.3% with m-Endo and 1.6% with m-ColiBlue24. However, the initial FPE data yielded a value of 43.6% with m-Endo and 52.0% with m-ColiBlue24. Because of these high values, overall agreement was only 77.2% for m-Endo and 73.6% for m-ColiBlue24. When TC isolates which had initially been categorized as noncoliforms were further analyzed by API20E, it was found that 14.6% of the putative noncoliforms on m-Endo actually belonged to one of the four classical TC genera. Similarly, 25.2% of putative noncoliforms on m-ColiBlue24 were actually TC. When these data were taken into account, the TC recoveries on m-ColiBlue24 and m-Endo were not significantly different. As a possible further improvement, use of the oxidase test, either in a standard or accelerated form, as a secondary screen for TC colonies on m-ColiBlue24 was explored. This increased the overall agreement slightly to 90.8%. These observations are in concurrence with abundant literature citing the difficulty of selectively isolating all species of the four classical coliform genera. Farmer et al. (12) list 28 species in these genera and Brenner lists 17 (4). It is difficult to recover all species, including stressed cells, while excluding all other gram-negative species.

Some information pertaining to the issue of FPE in other types of TC media is summarized here, beginning with five reports of results obtained with MF media. Cenci et al. (6) obtained an FPE value of 11.7% with m-Endo and 25.1% with MacConkey agar plus 4-methylumbelliferyl-β-D-galactopyranoside. LeChavallier et al. (22) reported 12.5% FPE and unidentified isolates with m-T7. Evans et al. (11) found that the FPE was 14.9% with m-Endo. Sartory and Howard (27), in describing their newly developed TC-*E. coli* medium, reported 29.8% FPE with m-LSB, including target colonies which proved to be oxidase positive or both acid and gas negative when transferred to Lactose-peptone-water. They also cited an FPE of 31.2% with m-LGA. Brenner et al. (5) reported that 15.4% of TC target colonies (fluorescent, nonblue) were *Serratia* spp. and *Hafnia alvei* and the average coliform verification rate for TC on MI was 77.6%.

As an illustration of TC FPE when the P-A format is used, Jacobs et al. (21) reported 26.3% of isolates were not classical TCs when a modification of Clark's formula was used. In testing LMX broth, Manafi (23) examined 771 cultures of coliforms and noncoliforms and reported that several species not typically regarded as TC gave positive galactosidase reactions. These included 31 of 34 *Serratia* sp. isolates, 14 of 22 *H. alvei* isolates, 9 of 9 *Aeromonas* sp. isolates, and 18 of 47 *Yersinia enterocolitica* isolates.

Several studies also provide estimates of TC FPE when media were used in a multiple tube fermentation format. Edberg et al. (10) reported an FPE of 16% by standard methods and 20% with Colilert. Evans et al. (11) reported an FPE of 5.4% with standard LTB and 12.3% with a modified version. Palmer et al. (25) found a 19% FPE with Colilert-Marine Water. Finally, Covert et al. (9) found that *Serratia* spp., *H. alvei*, *Vibrio fluvialis*, and *Aeromonas* spp. constituted 25.0% of isolates after 24 h and 40.8% of isolates after 28 h of incubation with Colilert. These four groups of noncoliforms constitute the main sources of FPE in most of the references cited above. These groups share important physiological characteristics with the four classical coliform genera. Some are also capable of causing disease. It has been suggested that the current definition of coliforms be modified to address this issue. The current United Kingdom drinking water regulations state that *Hafnia* spp., *Serratia* spp., and *Yersinia* spp. will be routinely isolated by standard methods for TC analysis (17). In conclusion, the FPE values for m-ColiBlue24 are similar to those obtained with numerous media for TC recovery and are consistent with evolving definitions of TC. This finding, plus its superior *E. coli* recovery, ease of use, and nominal cost make m-ColiBlue24 a useful medium for coliform analysis.

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