APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1981, p. 1144-1151
0099-2240/81/051144-08$02.00/0

Vol. 41, No. 5

Impact of Verification Media and Resuscitation on Accuracy of the Membrane Filter Total Coliform Enumeration Technique†

T. M. EVANS, RAMON J. SEIDLER,* AND M. W. LECHEVALLIER
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

Received 26 November 1980/Accepted 2 March 1981

Verification of membrane filter total coliform colonies was compared in lauryl tryptose broth, lactose broth, and *m*-LAC broth primary media and brilliant green-lactose-bile broth and EC broth secondary media. Verification in *m*-LAC broth yielded the greatest number of aerogenic isolates for both untreated surface water and drinking water samples. Verification in brilliant green-lactose-bile broth increased the number of false-negative reactions. At least 90% of the isolates aerogenic in primary verification media and anaerogenic in brilliant green-lactose-bile broth were representative of the coliform genera. The addition of a resuscitation step in the membrane filter technique did not yield greater numbers of verified coliforms per sample. Verification of both typical and atypical colonies in *m*-LAC broth resulted in a 10-fold increase in coliform numbers from untreated surface water. With drinking water, verification of both colony types resulted in an increase from less than 1 coliform per 100 ml to greater than 1/100 ml. A single-step verification in *m*-LAC broth is proposed as a more rapid and sensitive coliform verification procedure than the standard technique.

The monitoring of water supplies for the presence of total coliform bacteria is one means by which the sanitary quality of the supply is determined. The presence of any coliform bacteria in drinking water is an indication of a contaminated source, inadequate treatment, or post-treatment contamination and may be of sanitary significance (4, 7, 19). In untreated surface waters the number of coliforms present is an index of the overall sanitary quality.

Two procedures, the membrane filter (MF) and most-probable-number methods, are recognized for the enumeration of total coliforms in water samples (1, 23). Of the two techniques, the MF procedure is usually the method of choice (11, 17, 18) and potentially represents a more rapid, sensitive, and reproducible procedure than the most-probable-number technique (5).

Recently, several serious limitations of the MF technique have been recognized. Coliform bacteria have been detected by the most-probable-number technique in turbid drinking water samples when no typical coliforms were recovered by the MF technique (15). In addition, many bacteria which produce a reaction, i.e., green sheen, typical of coliform bacteria on the isolation medium have been anaerogenic in lactose-containing media (9-11, 14, 16, 20, 21). Several studies have indicated that the proportion of green-sheen colonies verifiable as coliforms can vary between 44 and 97% (9, 10, 16). This wide range in verification frequencies has resulted in the adoption of a verification scheme to be applied when the MF technique is used (2).

The verification procedure adopted by the U.S. Environmental Protection Agency consists of subculturing typical green-sheen colonies into lauryl tryptose broth (LTB) and examining the culture tubes for evidence of gas production after incubation for 24 to 48 h (2). The presence of a coliform organism is further confirmed by subculturing it into brilliant green-lactose-bile broth (BGLB). Only those colonies which give rise to gas production in both LTB and BGLB are considered coliforms. The necessity of verifying typical colonies essentially eliminates one of the advantages of the MF technique. Instead of obtaining the results of coliform analyses within 24 h, up to 5 days may be required to obtain an accurate verified count. In addition, the need for verification imposes a substantial burden for monitoring laboratories in terms of materials and labor required.

Any factor which impedes sheen development of coliform colonies would make the selection of colonies for verification difficult and would therefore influence the accuracy of the MF tech-

† Technical Paper no. 5767, Oregon Agricultural Experiment Station.

nique. One factor which could influence sheen development is the extent and type of nutrients which surround the coliform during initial incubation. An extended growth lag of coliforms on the membrane filter could occur as a result of physiological injury to coliforms. The addition of a pre-enrichment or resuscitation step has been used to partially alleviate coliform injury problems (20). A resuscitation step in the MF procedure could influence the success of colony verification by either selecting for certain coliforms or enhancing sheen development.

Any factor which impedes gas production by coliforms in the verification procedure would also influence the accuracy of the MF technique. The composition of lactose-containing media is known to influence gas production by coliforms (3, 8). In addition, gas production by certain coliforms is inhibited or reduced in BGLB (13).

The present study was conducted to determine the influence of verification media and resuscitation on the number of MF typical colonies which produce gas during verification. LTB, LB, and an *m* Endo-based broth (*m*-LAC) were compared as primary verification media. The need for and use of BGLB as a secondary verification medium were also examined. The occurrence of false-negative results due to the lack of sheen production by coliforms was also assessed.

MATERIALS AND METHODS

Study area and sample collection. Samples were collected from several untreated surface water sources and a finished drinking water supply which serves an Oregon coastal community of 14,000 residents. Chlorination is the only treatment applied to the raw water before entry into the drinking water distribution system.

Water samples were collected in sterile, 4-liter polypropylene containers with (drinking water) or without (untreated surface water) added sodium thiosulfate. Samples were placed on ice and transported back to the laboratory within 3 h after collection and analyzed within 7 h of collection.

Microbiological techniques. Two MF techniques were used for the enumeration of total coliforms. The standard and resuscitation MF techniques were conducted according to standard procedures (1), using Gelman GN-6 membranes (pore size, 0.45 μm) and *m* Endo agar LES (Difco lot no. 663038 and 663465). Duplicate volumes of 250 and 100 ml of drinking water and 25-, 10-, and 1-ml volumes of untreated surface water were routinely analyzed. In the resuscitation technique, MF were placed onto sterile pads (Gelman no. 66025) saturated with sterile LTB (Difco lot no. 663634, 663637, 666376, and 668945) and incubated at 35°C for 1.5 to 2 h. The MF were then transferred to *m* Endo agar LES and incubated for an additional 22 to 22.5 h.

Those colonies described by *Standard Methods for the Examination of Water and Waste Water* (1) as being typical were counted with the aid of a dissecting microscope, and at least 30% of the colonies were submitted to the verification scheme. Also, any colony with a questionable green sheen was included in the typical colony count and verified. In some drinking water samples where the numbers of typical colonies were less than 10 per plate, colonies were picked from replicate plates. In this case, when possible, a total of 10 colonies were verified. In additional experiments, atypical colonies (dark red, pink, or clear and without any evidence of a sheen) were selected from a limited number of plates for verification. Atypical coliform counts were included in the MF count in only those experiments which were designed to determine the percentage of false-negative coliform colonies on an MF.

The verification scheme consisted of picking colonies from the MF and inoculating slants of tryptic soy-yeast extract agar. The slants were prepared by supplementing tryptic soy broth (Difco lot no. 605317, 663068, and 670390) with 0.3% yeast extract (Difco lot no. 668517) and 1.5% agar. After a 24 h of incubation at 35°C, growth from the slant was inoculated into the various primary verification media. This procedure, as opposed to direct inoculation of colony growth from the MF, ensured that all verification media were inoculated with suitable numbers of viable cells to uniformly allow growth in the broth verification media in 24 h. The subculture to tryptic soy-yeast extract agar slants was especially critical for obtaining suitable amounts of inoculum for those pinpoint sheen colonies occasionally appearing on the MF. The number of colonies which produced gas when directly inoculated into the verification media was compared with the number which produced gas when first subcultured onto tryptic soy-yeast extract agar slants. The results indicated that the probability of gas production by a colony was not influenced by the colony's being cultured first on tryptic soy-yeast extract agar.

Each typical colony after being subcultured onto a tryptic soy-yeast extract agar slant was inoculated into each of three different primary verification media. LTB, LB (Difco lot no. 652242, 662331, and 671048), and *m*-LAC broth. The composition of *m*-LAC broth was based on the formulation of *m* Endo agar LES (1) and consisted of: 1.2 g of yeast extract (Difco lot no. 668517), 3.7 g of Casitone (Difco lot no. 537023), 3.7 g of peptone (Difco lot no. 670999), 7.5 g of tryptose (Difco lot no. 666147), 9.4 g of lactose (Baker lot no. 714399), 3.3 g of K_2HPO_4 (Baker lot no. 635081), 1.0 g of KH_2PO_4 (Baker lot no. 715107), 3.7 g of NaCl (Sigma lot no. 58C-0274), 0.1 g of sodium deoxycholate (Sigma lot no. 96C-0474), and 0.05 g of sodium lauryl sulfate (Sigma lot no. 20F-0424), all dissolved in 1 liter of distilled water. The medium was adjusted to pH 7.2 and autoclaved at 121°C for 15 min. The three fermentation broths were examined for evidence of gas production after incubation at 35°C for 24 and 48 h. All gas-postive tubes were subcultured into two secondary verification media: BGLB (Difco lot no. 666632 and 669678) and EC broth (Difco lot no. 641057 and 651945). The BGLB and EC broth tubes were examined for evidence of gas production after incubation at 35°C for 24 and 48 h.

The number of verified coliforms per 100 ml of sample was calculated by multiplying the percentage of colonies aerogenic in the verification media by the average number of typical colonies on the duplicate plates.

Identification of coliform isolates. Aerogenic isolates recovered by each of the two MF techniques were selected for identification. Ten aerogenic isolates were selected per untreated surface water sample, and, where possible, an equal number were selected from each drinking water sample. None of the aerogenic isolates were found to be gram positive. Isolates identified were aerogenic in both primary (LTB and *M*-LAC) and secondary (BGLB) media or aerogenic in primary media and anaerogenic in BGLB. Cultures were streaked for isolation onto *m* Endo agar LES before being identified according to a scheme previously described (12). All atypical aerogenic colonies were also identified.

Quality control and statistical comparisons. A quality assurance program as outlined in *Microbiological Methods for Monitoring the Environment* (2) was used throughout the course of this study. Performances of media and sterility controls were determined on a per lot or per batch basis. Materials used during each sampling event were checked for sterility. The temperatures of autoclaves and incubators were monitored on a per use basis.

Statistical comparisons were made on the basis of the paired *t*-test, using logarithmically transformed data (22).

RESULTS

The results of this study are based on water samples collected over a 1-year period. The MF analyses were conducted on 21 untreated surface water samples (all of which were coliform positive) and 120 drinking water samples (36 of which were coliform positive). Typical green-sheen colonies (550 from untreated surface water and 625 from drinking water) were submitted to both primary (LTB, LB and *m*-LAC broth) and secondary (BGLB and EC broth) verification media.

Affect of verification media on the geometric mean coliform number per sample. The number of typical colonies found to be aerogenic in the verification media was used to determine a verified coliform count per 100 ml of sample. None of the aerogenic isolates recovered by the MF technique were found to be gram positive or oxidase positive.

The impacts of resuscitation, verification media, and water source on the geometric mean numbers of coliforms recovered are presented in Table 1. The greatest numbers of coliforms in untreated surface water were obtained by the MF technique when verification was performed in *m*-LAC broth. *m*-LAC broth also demonstrated the greatest percentages of aerogenic typical colonies. Verification in *m*-LAC broth yielded significantly greater mean numbers of coliforms than did either LTB or LB as determined by the *t* statistic (first three comparisons, Table 2). Both LTB and LB yielded comparable numbers of aerogenic isolates and mean coliform numbers (Tables 1 and 2), but *m*-LAC broth counts were about 50% greater.

TABLE 1. *Impacts of verification media and resuscitation on the geometric mean numbers of coliforms recovered from untreated surface water and drinking water supplies*

Primary verification medium	Secondary verification medium	Geometric mean no. of total coliforms/100 ml of:			
		Untreated surface water		Drinking water[a]	
		No resuscitation	Resuscitation	No resuscitation	Resuscitation
LTB	None	150	160	1.7	2.1
	BGLB	90	67	1.4	1.4
	EC broth	140	130	1.7	1.7
LB	None	140	160	2.5	2.4
	BGLB	97	78	1.6	1.7
	EC broth	110	130	1.4	1.9
m-LAC broth	None	210	220	3.5	2.8
	BGLB	120	110	1.9	2.0
	EC broth	180	170	2.5	2.5

[a] Means were calculated only for those samples in which the numbers of typical colonies were ≥1/100 ml.

Secondary verification of MF typical colonies in either BGLB or EC broth reduced the geometric mean number of coliforms obtained from untreated surface water samples (Table 1). EC broth yielded a significantly greater mean coliform number than did BGLB when the primary verification medium was either LTB or *m*-LAC broth, but not with LB (Tables 1 and 2). The two-step verification in *m*-LAC broth and BGLB yielded significantly greater mean numbers of coliforms than did the two-step verification in LTB and BGLB.

The greatest geometric mean numbers of aerogenic coliforms were obtained from drinking water when typical colonies were verified in *m*-LAC broth only (Table 1). Verification in *m*-LAC broth resulted in 2 times or 1.4 times, respectively, the mean numbers obtained with LTB or LB (Table 1). These differences were significant at the 0.1% level (Table 2). The mean coliform numbers obtained with either LTB or LB verification were not significantly different from each other (Table 2).

Secondary verification in BGLB resulted in significant reductions in geometric mean coliform counts from those obtained with LTB, LB or *m*-LAC broth verification for contaminated drinking water samples (Table 1). Verification in EC broth also resulted in reduced coliform

TABLE 2. *Statistical comparisons based on the impacts of verification media and resuscitation on recovery of coliforms*[a]

Comparison	Untreated surface water				Drinking water			
	No resuscitation		Resuscitation		No resuscitation		Resuscitation	
	t[b]	P	t	P	t	P	t	P
LTB vs LB	+0.287	>0.5	+0.285	>0.5	−1.999	0.055	+0.037	>0.5
LTB vs *m*-LAC	−5.188	<0.001	−3.837	0.001	−4.759	<0.001	−2.465	0.021
LB vs *m*-LAC	−2.175	0.043	−3.643	0.002	−3.926	<0.001	−0.978	0.336
LTB-BGLB vs LB-BGLB	−0.503	>0.5	−0.495	>0.5	−1.483	0.155	−0.385	>0.5
LTB-BGLB vs *m*-LAC-BGLB	−2.984	0.008	−1.722	0.100	−2.835	0.008	−2.737	0.011
LB-BGLB vs *m*-LAC-BGLB	−1.136	0.281	−1.508	0.209	−1.774	0.089	−1.331	0.200
LTB-BGLB vs LTB-EC	−4.023	<0.001	−2.371	0.029	−1.906	0.070	−1.722	0.097
LB-BGLB vs LB-EC	−1.730	0.097	−2.062	0.053	+0.235	>0.5	−1.078	0.303
m-LAC-BGLB vs *m*-LAC-EC	−4.086	0.005	−3.435	0.003	−2.504	0.020	−2.308	0.029

[a] Statistical comparisons based on paired two-tailed t-tests performed on logarithmically transformed data.
[b] The sign of the t value indicates whether the first member of the comparison (+) or the second member (−) gave a higher mean number.

numbers. EC broth did yield significantly greater mean coliform numbers than did BGLB when the primary verification medium was *m*-LAC broth (Table 2).

The addition of a resuscitation step to the MF analyses of both untreated surface water and drinking water samples did not result in an increase in the number of typical colonies which appeared on the MF or in greater mean coliform numbers being obtained from any of the verification procedures (P values all > 0.1) (Table 1).

Colony verification frequency on a per sample basis. The differential recovery of aerogenic isolates in primary versus secondary media as well as medium composition influenced the percentage of colonies which were verified on a sample-to-sample basis (Fig. 1). Thus, with surface water samples and the standard verification procedure (LTB and BGLB), 80% or more of the typical colonies were verified in only 18% of the samples examined. Single-step verification in either LTB or *m*-LAC broth increased the detection of aerogenic coliforms on a per sample basis, with *m*-LAC broth yielding greater verification frequencies than LTB. Verification in *m*-LAC broth resulted in 80% or more of the typical colonies being verified in 50% of the samples. This pattern was similar to that of the drinking water samples. Using the standard verification procedure, 80% of the typical colonies were aerogenic in only 14% or less of the samples. However, 80% of the typical colonies were verified in 60% of the samples with the single-step *m*-LAC broth procedure. The use of the resuscitation step did not influence colony verification on a sample-to-sample basis.

Identification of aerogenic coliform isolates. Since verification in *m*-LAC broth yielded a significantly higher verified count than that obtained with LTB, aerogenic isolates were identified to determine whether *m*-LAC broth selected for a different group of coliforms than that selected for by LTB. Approximately 300 untreated surface water aerogenic isolates and 350 drinking water aerogenic isolates were identified. The number of isolates identified for each water type included an equal number of isolates recovered by each of the MF techniques. *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella* comprised 95% of the aerogenic coliforms from surface water and drinking water. The lactose-fermenting biotypes of *Yersinia enterocolitica* and *Hafnia alvei* comprised the remaining aerogenic isolates. No differences were observed in the component genera which were aerogenic in both LTB and *m*-LAC broth. Also, the component coliform genera aerogenic in the standard LTB-BGLB verification scheme did not differ from those aerogenic in *m*-LAC broth and BGLB.

The MF resuscitation procedure produced few changes in the percentages of component coliform genera aerogenic in the verification media. The only observed difference as a result of resuscitation was the increase in aerogenic *Y. enterocolitica* and *H. alvei* to 10% of the isolates recovered from contaminated drinking water samples.

Citrobacter was the most common genus to be aerogenic in primary (LTB or *m*-LAC broth) media and anaerogenic in secondary media (Fig. 2). *Enterobacter* and *Klebsiella* were also found, but at lower frequencies than *Citrobacter*, to be aerogenic in only primary media. Less than 2% of the *Escherichia coli* isolates were anaerogenic in BGLB (Fig. 2). This pattern was found for both types of water samples examined and both MF techniques used. Lactose-fermenting bio-

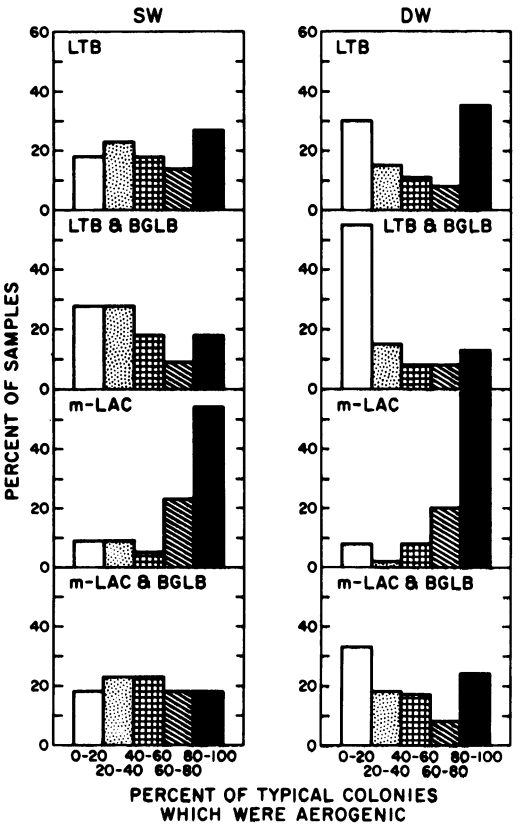

FIG. 1. *Percentages of typical sheen colonies which were aerogenic in various verification media on a sample-to-sample basis. Typical colonies isolated by the MF technique (no resuscitation) from untreated surface water (SW) and drinking water (DW) samples were verified in primary (LTB or m-LAC broth) and secondary (BGLB) media. For example, with SW, 80% or more of the typical colonies were aerogenic in LTB in only 25% of the samples examined.*

types of *Y. enterocolitica* and *H. alvei* comprised less than 10% of the isolates anaerogenic in BGLB.

Citrobacter was the most common genus to be aerogenic in *m*-LAC broth and anaerogenic in LTB (Fig. 2). This was evident for both untreated surface water and contaminated drinking water samples examined by either MF technique. Other isolates aerogenic in only *m*-LAC broth were identified as *Enterobacter* and *Klebsiella* for untreated surface water samples and as *Enterobacter* for drinking water samples. The lactose-fermenting biotypes of *Y. enterocolitica* and *H. alvei* comprised no greater than 10% of the *m*-LAC broth uniquely aerogenic isolates obtained from either untreated surface water or contaminated drinking water.

Verification of atypical MF colonies. Both typical and atypical coliform colonies (no resuscitation step) from five untreated surface water samples were submitted to the verification scheme. MF colonies which displayed any green sheen were considered typical. Colonies which appeared dark red, pink, or clear and lacked any evidence of a sheen were classified as atypical. Of the 223 atypical coliform colonies examined, 53 were aerogenic in *m*-LAC broth and 30 were aerogenic in LTB. The 53 isolates aerogenic in *m*-LAC broth were identified as *Citrobacter* (35%), *Enterobacter* (34%), *Klebsiella* (17%), *Y. enterocolitica* and *H. alvei* (12%), and *Escherichia* (2%). The standard MF technique (verification of typical colonies in LTB and BGLB) indicated that the geometric mean coliform number per sample was 34/100 ml. Verification

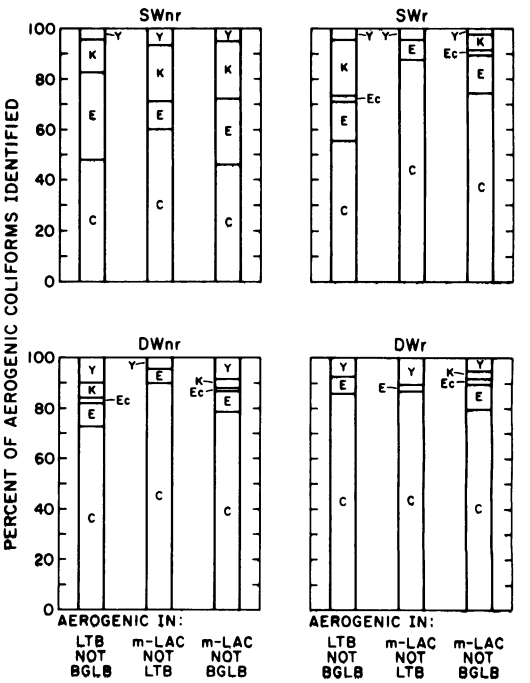

FIG. 2. *Coliform genera found to be aerogenic in various primary media (LTB or m-LAC broth), anaerogenic in secondary BGLB, or anaerogenic in LTB but aerogenic in m-LAC broth. Total coliforms were recovered from untreated surface water (SW) or contaminated drinking water (DW) samples by the MF technique with (SWr, DWr) or without (SWnr, DWnr) resuscitation in parallel analyses. For example, the figure illustrates, with SWnr, that Citrobacter (C) comprised 48%, Enterobacter (E) comprised 34%, Escherichia (Ec) comprised 0%, Klebsiella (K) comprised 12%, and Y. enterocolitica or H. alvei or both (Y) comprised 5% of the coliform genera aerogenic in LTB and anaerogenic in BGLB.*

of both typical and atypical coliform colonies in *m*-LAC broth yielded a geometric mean aerogenic coliform number of 350/100 ml.

The effect of verifying both typical and atypical coliform colonies on the accuracy of the MF technique was also determined on 14 contaminated drinking water samples. Of the 202 atypical colonies examined, 51 were aerogenic in *m*-LAC broth and 47 were aerogenic in LTB. The 51 isolates aerogenic in *m*-LAC broth were identified as *Citrobacter* (44%), *Enterobacter* (39%), *Klebsiella* (6%), and *Y. enterocolitica* and *H. alvei* (11%). The standard MF verification technique yielded a geometric mean total coliform number for these 14 samples of 0.8/100 ml, whereas verification of both typical and atypical colonies in *m*-LAC broth indicated that the aerogenic mean coliform number was 1.2/100 ml.

DISCUSSION

The only accepted indication of the sanitary quality of potable water supplies is the absence of the total coliform bacterial group based on a monthly average. Since the presence of any coliform in potable drinking water supplies may represent contamination at the source, inadequate treatment, or post-treatment contamination (4, 19), coliform detection techniques should be as sensitive as the state of the art permits (15).

The accuracy and sensitivity of the MF technique are greatly influenced by the efficiency of the verification procedure. The procedure of primary verification in LTB and secondary verification in BGLB was designed to eliminate false-positive reactions, i.e., typical green-sheen colonies resulting from noncoliform bacteria (2, 16). The previously recognized causes of false-positive reactions were sheen production by slow lactose fermenters (gas production between 48 and 96 h) (16), sheen production by gram-positive bacteria (16), and sheen production by synergistic reactions between two lactose nonfermenters (21).

The greater frequency of gas production by typical colonies in *m*-LAC broth as compared with LTB or LB demonstrated the effect of medium composition on gas production and efficiency of verification. The superiority of *m*-LAC broth to either LB or LTB may result from the inclusion of nutritional factors which enhance the extent of growth or rate of gas production, or both, by the isolates. The composition of lactose-containing media is known to influence both the rate and the amount of gas production by coliform bacteria (3, 8).

The use of *m*-LAC broth to demonstrate gas production by typical colonies may have resulted in the coliforms previously reported as slow lactose fermenters in LTB or LB (16) being able to produce gas within the 48-h time interval stipulated by the *Standard Methods* (1) definition of a coliform. The isolates aerogenic in *m*-LAC broth but anaerogenic in LTB were identified as genera (*Citrobacter*, *Enterobacter*, and *Klebsiella*) representative of the coliform group (6). Therefore, the use of *m*-LAC broth as a primary verification medium would increase both the accuracy and the sensitivity of the MF total coliform enumeration technique.

Secondary verification in BGLB or EC broth of isolates aerogenic in LTB or *m*-LAC broth reduced verification frequency by up to 40% with drinking water samples. The use of EC medium yielded a greater verification frequency than that found with BGLB, but still resulted in a reduced verification frequency compared with LTB and *m*-LAC broth. At least 90% of the isolates aerogenic in primary verification media and anaerogenic in BGLB were identified as *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*. None of the isolates found to be aerogenic in primary media and nonaerogenic in BGLB were gram positive or oxidase positive. The inclusion of selective agents (sodium lauryl sulfate in both LTB and *m*-LAC broth and sodium deoxycholate in *m*-LAC broth) in the primary verification media may explain why gram-positive bacteria were not recovered. The absence of lactose-fermenting *Aeromonas* may be due to low water temperatures or a lack of proper nutrients needed for this genus to be prevalent in the environment. *Aeromonas* has been reported by others to interfere with coliform detection by the MF technique when certain waters have been examined (18). Therefore, secondary verification in BGLB is not necessary to demonstrate that MF typical colonies are coliforms. The inclusion of BGLB in the standard verification scheme serves only to decrease the sensitivity of the MF technique and increase by 24 to 48 h the time necessary to obtain the results of an analysis.

The addition of a resuscitation step to the MF technique did not increase the number of typical colonies recovered from untreated surface water or chlorinated drinking water samples, nor did it enhance the percentage of verification of typical colonies. Therefore, the inclusion of a resuscitation step in the MF procedure provided no distinct advantage, at least in this study, in terms of increased coliform recovery from water samples. Other investigators have reported that resuscitation enhances total coliform recovery from surface water samples (20). Since the water samples examined in this study were obtained

from one geographical region, additional information is needed to determine whether a preenrichment step in the MF technique results in better coliform recovery. In addition, the effect of verification media on the number of coliforms recovered by the resuscitation MF technique should also be examined.

The enumeration of coliforms by the MF technique depends on the utilization of media that will produce a consistent, easy-to-recognize differentiation of coliforms from other organisms in a water sample. The number of false-negative results (atypical colonies aerogenic in lactose-containing media) in the MF technique with Endo-type media has usually been determined by verification in either LB or LTB (14). Fifield and Schaufus (14) reported in their study of surface water samples that 6.4% of the atypical colonies represented false-negative results. Verification of atypical colonies in *m*-LAC broth indicated that at least 24% of the atypical colonies recovered from both untreated surface water and drinking water represented false-negative results. Over 85% of the atypical aerogenic colonies were identified as *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*. In the 14 contamined drinking water samples tested, the geometric mean number of coliforms changed from less than 1 to over 1/100 ml when both typical and atypical colonies were tested for gas production in *m*-LAC broth. Therefore, atypical colonies which appear on membranes incubated on *m* Endo agar LES do not necessarily represent negative results, but they may represent a failure of the MF technique to detect coliforms in contaminated samples.

An MF technique with verification of typical colonies in only *m*-LAC broth would provide definite advantages over the current standard technique. A single-step verification in *m*-LAC broth of MF typical colonies yielded, in the current study, 2.3- and 2.5-fold increases, respectively, for untreated surface water and drinking water samples over standard verification in LTB and BGLB. A greater number of false-positive reactions would not result with *m*-LAC broth verification, and the component coliform genera recovered would be comparable to those obtained by the standard technique.

In conclusion, a single-step verification of MF typical colonies would provide a more rapid and sensitive technique than is currently practiced. The feasibility of using *m*-LAC verification media should be determined on a number of different water types from several geographic locations. Such an investigation would determine the efficacy of a single-step verification procedure in eliminating false-positive results in the MF technique. In addition, the occurrence of atypical coliform colonies on MF filters should be more closely examined, especially with drinking water, to determine the efficacy of the differential reaction occurring on *m* Endo agar LES.

ACKNOWLEDGMENT

This research was supported by cooperative agreement CR806287 from the U.S. Environmental Protection Agency, Drinking Water Research Division.

LITERATURE CITED

1. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Inc., Washington, D.C.
2. **Bordner, R., and J. Winter (ed.).** 1978. Microbiological methods for monitoring the environment. U.S. Environmental Protection Agency, Cinicinnati.
3. **Chambers, C. W.** 1950. Relationship of coliform bacteria to gas production in media containing lactose. Public Health Rep. **65**:619–627.
4. **Clark, H. F., and P. W. Kabler.** 1964. Reevaluation of the significance of coliform bacteria. J. Am. Water Works Assoc. **56**:931–936.
5. **Clark, H. F., P. W. Kabler, and E. E. Geldreich.** 1957. Advantages and limitations of the membrane filter procedure. Water Sewage Works **104**:385–387.
6. **Clark, J. A., and J. E. Pagel.** 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Microbiol. **23**:465–470.
7. **Craun, G. F.** 1978. Impact of the coliform standard on the transmission of disease, p. 21–35. *In* C. W. Hendricks (ed.), Evaluation of the microbiology standards for drinking water. U.S. Environmental Protection Agency, Washington, D.C.
8. **Darby, C. W., and W. L. Mallmann.** 1939. Studies on media for coliform organisms. J. Am. Water Works Assoc. **31**:689–706.
9. **Davenport, C. V., E. B. Sparrow, and R. C. Gordon.** 1976. Fecal indicator bacteria persistence under natural conditions in an ice-covered river. Appl. Environ. Microbiol. **32**:527–536.
10. **Dutka, B. J.** 1973. Coliforms are an inadequate index of water quality. J. Environ. Health. **36**:39–48.
11. **Dutka, B. J., and S. E. Tobin.** 1976. Study on the efficiency of four procedures for enumerating coliforms in water. Can. J. Microbiol. **22**:630–635.
12. **Evans, T. M., M. W. LeChevallier, C. E. Waarvick, and R. J. Seidler.** 1981. Coliform species recovered from untreated surface water and drinking water by the membrane filter, standard, and modified most-probable-number techniques. Appl. Environ. Microbiol. **41**: 657–663.
13. **Evans, T. M., C. E. Waarvick, R. J. Seidler, and M. W. LeChevallier.** 1981. Failure of the most-probable-number technique to detect coliforms in drinking water and raw water supplies. Appl. Environ. Microbiol. **41**: 130–138.
14. **Fifield, C. W., and C. P. Schaufus.** 1958. Improved membrane filter medium for the detection of coliform organisms. J. Am. Water Works Assoc. **50**:193–196.
15. **Geldreich, E. E., M. J. Allen, and R. H. Taylor.** 1978. Interferences to coliform detection in potable water supplies, p. 13–20. *In* C. W. Hendricks (ed.), Evaluation of the microbiology standards for drinking water. U.S. Environmental Protection Agency, Washington, D.C.
16. **Geldreich, E. E., H. L. Jeter, and J. A. Winter.** 1976. Technical considerations in applying the membrane filter procedure. Health Lab. Sci. **4**:113–125.

17. **Geldreich, E. E., and H. Kennedy.** 1978. The cost of microbiological monitoring, p. 1-20. *In* Proceedings of the National Science Foundation Seminar, Philadelphia, Pa. National Science Foundation, Washington, D.C.
18. **Grabow, W. O. K., and M. du Preez.** 1979. Comparison of *m*-Endo LES, MacConkey, and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl. Environ. Microbiol. **38**:351-358.
19. **Kabler, P. W., and H. F. Clark.** 1960. Coliform group and fecal coliform organisms as indicators of pollution in drinking water. J. Am. Water Works Assoc. **52**:1577-1579.
20. **McCarthy, J. A., J. E. Delany, and R. J. Grasso.** 1961. Measuring coliforms in water. Water Sewage Works **108**:238-243.
21. **Schiff, L. J., S. M. Morrison, and J. V. Mayeux.** 1970. Synergistic false-positive coliform reaction on M-Endo MF medium. Appl. Microbiol. **20**:778-781.
22. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods. The Iowa State University Press, Ames.
23. **U.S. Environmental Protection Agency.** 1975. National interim primary drinking water regulations. Fed. Regist. **40**:59566-59588.