APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1979, p. 229-236
0099-2240/79/08-0229/08$02.00/0

Vol. 38, No. 2

Rapid Seven-Hour Fecal Coliform Test

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Received for publication 18 May 1979

A rapid 7-h fecal coliform (FC) test for the detection of FC in water has been developed. This membrane filter test utilizes a lightly buffered lactose-based medium (m-7-h FC medium) combined with a sensitive pH indicator system. FC colonies appeared yellow against a light purple background after incubation at 41.5°C for 7 to 7.25 h. Comparison of FC test results showed that the mean verified FC count ratio (7-h FC count/24-h FC count) for surface water samples was 1.08. The mean FC count ratio (7-h FC count/24-h FC count) for unchlorinated wastewater ranged from 1.95 to 5.05. Verification of yellow FC colonies from m-7-h FC medium averaged 97%. Data from field tests on Lake Michigan bathing beach water samples showed that unverified 7-h FC counts averaged 96% of the 24-h FC counts. The 7-h FC test was found to be suitable for the examination of surface waters and unchlorinated sewage and could serve as an emergency test for detection of sewage or fecal contamination of potable water.

The need for rapid determination of the sanitary quality of water has been cited most often in relation to testing emergency or temporary potable water supplies, recreational waters, and shellfish-growing waters subject to sewage pollution. The usefulness of a rapid bacteriological test is determined by several important factors including ease of use, accuracy and sensitivity, economy of use, and test results that provide good correlation with results obtained by using accepted standard procedures as outlined in *Standard Methods for the Examination of Water and Wastewater* (1). Approaches to fulfilling the need for a rapid sanitary indicator test have included the development of sophisticated methods that require special instrumentation, reagents, etc. However, methods that require skilled personnel and sophisticated equipment may not be adaptable to authentic emergency situations. Therefore, the most suitable approach appears to be that of modifying conventional coliform methodology so that the results can be obtained within a much shorter time than the usual 24- to 48-h period. Van Donsel et al. (D. J. Van Donsel, R. M. Twedt, and E. E. Geldreich, Bacteriol. Proc., p. 25, 1969) used a gradient temperature incubation block to determine that 41.5°C was the optimum incubation temperature for quantitation of fecal coliforms (FC) in 7 h. Coupling this optimum incubation temperature with a medium that contains lactose and mannitol and a sensitive acid-base indicator system permitted quantitation of FC in less than an 8-h work day. In this paper, data are presented on the final development and field testing of the membrane filter 7-h FC test.

MATERIALS AND METHODS

m-7-h FC medium. The medium formulation and preparation instructions are shown in Table 1. Prepare the medium in bulk dehydrated quantities by grinding appropriate quantities of the ingredients (except agar) with a morter and pestle or in a ball mill. After thorough grinding and mixing, dry the medium overnight in a drying oven at 50°C. The medium should be placed in a clean, dry bottle, sealed tightly, and stored in a warm, dry place or stored in a desiccating chamber with fresh desiccant. The membrane filter medium (m-7-h FC medium) is prepared by using 3.14 g of dehydrated m-7-h FC medium per 100 ml of distilled water. After dissolving the ingredients, the pH should be checked and adjusted to 7.3. Add 1.5 g of agar per 100 ml of m-7-h FC medium after pH adjustment. Alternatively, prepare bulk m-7-h FC medium in 1-liter volumes by dissolving all the ingredients except agar in 1 liter of distilled water, adjust the pH to 7.3, and filter sterilize the solution. Refrigerate the stock medium and use as needed to prepare 100-ml volumes of the m-7-h FC medium. Add 1.5 g of agar per 100 ml of medium and heat to boiling to dissolve the agar. Dispense 4- to 6-ml volumes of m-7-h FC medium into tight-lid petri dishes; prepared plates can be stored and refrigerated (2 to 8°C) for up to 2 weeks, provided excessive drying does not occur.

Sample sources and sample collection. Water samples were collected from 18 sources in the Cincinnati, Ohio, vicinity. The water sources included surface runoff, creeks, rivers, lakes, and a drinking water reservoir.

Water samples were collected from about 10 to 15 cm below the surface in sterile, 1-liter, polypropylene

TABLE 1. *m-7-h FC medium formulation*[a]

Component	Amt
Proteose peptone no. 3[b]	5.0 g
Yeast extract	3.0 g
Lactose	10.0 g
D-Mannitol	5.0 g
Sodium chloride	7.5 g
Sodium lauryl sulfate	0.2 g
Sodium deoxycholate	0.1 g
Bromocresol purple, free acid	0.35 g
Phenol red, water soluble	0.3 g
Agar	15.0 g
Distilled water	1,000.0 ml
Final pH[c]	7.3

[a] Commercial medium available from Difco (RX 184734) or BBL (no. 95066) by special request.

[b] All ingredients manufactured by Difco Laboratories, except sodium chloride, bromocresol purple, and phenol red, which were manufactured by Fisher Scientific Co.

[c] Add all ingredients except agar to the distilled water, mix to dissolve, and adjust pH to 7.3 by using sterile 0.1 N NaOH or 0.1 N HCl as needed. After pH adjustment, add agar, heat medium to boiling to dissolve agar, cool to 45 to 50°C, and dispense 5- to 6-ml volumes into tight-lid petri dishes, 50 by 12 mm or 60 by 12 mm in diameter.

bottles by the grab sample technique (1). All samples were transported to the laboratory and examined initially within 2 h after collection by the *Standard Methods* (1) membrane filter FC procedure to establish FC concentration before parallel test comparison. The samples were refrigerated overnight and re-examined by the 7-h FC test and the standard 24-h FC membrane filter procedure in parallel.

Procedure. The samples were filtered with 0.45-μm-pore-size, 47-mm-diameter membrane filters (Millipore HAWG), and the membrane filters were placed on m-7-h FC agar and on m-FC agar (1). Gelman GN-6 and Millipore HCWG membrane filters were tried, but because of the larger surface pore openings colony size and color were not well developed in 7 h. Consequently, FC colonies were more difficult to count on the Gelman GN-6 and Millipore HCWG membrane filters than on the Millipore HAWG membrane filters. Three replicate filtrations of each sample volume or dilution were prepared. The tight-lid petri dishes were firmly closed, sealed in Whirl-Pak bags, inverted, submerged in a 41.5 ± 0.2°C water bath, and incubated for 7 to 7.25 h. After the plates were removed from the water bath, the membranes were examined with a binocular wide-field dissecting microscope (10 to 15×), and all yellow colonies, both pale and bright yellow, were counted as FC. Water sample volumes or dilutions that resulted in 20 to 60 FC colonies per membrane filter were used. A Bausch and Lomb fluorescent microscope illuminator provided best illumination of the colonies for examination and counting. After completion of colony counts, at least 10 yellow colonies from one replicate plate were picked into lauryl tryptose broth and incubated at 35 ± 0.5°C for 24 ± 2 h. FC verification was completed by inoculating EC broth tubes from gas-positive lauryl tryptose broth tubes and incubating the EC tubes at 44.5 ± 0.2°C for 24 ± 2 h. Blue-colored FC colonies from m-FC medium were verified by the same procedure used to verify the yellow colonies from the m-7-h FC medium. Nonyellow colonies (clear, colorless and purple) were also picked and carried through the verification procedure to evaluate medium selectivity.

Field testing. Personnel from the Evanston-Northshore Health Department, Evanston, Ill., and the Washington Suburban Sanitary Commission, Hyattsville, Md., field tested the m-7-h FC procedure for monitoring FC populations in bathing beach waters and in unchlorinated wastewaters, respectively. Personnel in the Evanston-Northshore Health Department used laboratory-prepared m-7-h FC medium and also trial batches of Difco and Baltimore Biological Laboratories (BBL) m-7-h FC medium. m-7-h FC medium used at the Washington Suburban Sanitary Commission was prepared from individual ingredients.

RESULTS

Mean verified FC counts determined by both the standard 24-h FC procedure and by the 7-h FC procedure are shown in Table 2. The mean ratio, 7-h FC count per 100 ml/m-FC count per 100 ml (7-h FC/24-h FC) ranged from 0.55 to 2.00; the mean ratio of all samples was 1.08. A statistical analysis of the ratios (7-h/24-h) for all

TABLE 2. *Comparison of verified FC results obtained from the standard 24- and the 7-h membrane filter procedures for the examination of water samples from various sources*

Sample source	No. of samples	Mean 24-h FC/100 ml	Mean 7-h FC/100 ml	Mean ratio 7-h FC/24-h FC
Rivers and creeks				
Ohio River	32	4,400	4,000	0.92
Mill Creek	8	2,800	7,000	2.00
Little Miami R.	2	680	540	0.82
Duck Creek	8	1,200	1,000	0.95
Sycamore Creek	10	240	240	1.12
Clough Creek	1	3,800	7,500	1.97
Surface runoff				
Tusculum	2	240	200	0.81
Lakes and ponds				
Cowan Lake	3	76	83	1.13
Stonelick Lake	3	88	99	1.10
Devou Park	4	56	28	0.55
Winton Woods	5	96	91	0.90
Sharon Woods	6	91	120	1.03
Walton Reservoir	5	90	110	1.46
Tupper Pond	3	500	460	0.85
All sources	92	2,000	2,300	1.08[a]

[a] Mean of all sample ratios.

sources with three or more samples is shown in Table 3. The high sample variance observed for the Mill Creek samples resulted from two samples for which the 7-h/24-h ratio was very high (3.06 and 4.22). The Walton Reservoir mean variance was high due to the combination of a low number of samples and some extreme 7-h/24-h ratios. Increased numbers of samples from both the Mill Creek and Walton Reservoir Sources would probably have reduced this variance markedly.

Verification of yellow FC colonies picked from the m-7-h FC medium is shown in Table 4. The percent verification for all samples averaged 97.3%. Verification of blue FC colonies from the 24-h procedure (m-FC agar) ranged from 93 to 100% (data not shown) for the same samples. Table 5 shows verification percentages for nonyellow colonies picked from the m-7-h FC medium. These data indicate that many of the nonyellow colonies were also verified as FC by standard verification procedures. These results suggest that 41.5°C incubation allowed recovery of stressed FC that failed to repair and grow when incubated at 44.5°C in the standard FC test. The stressed organisms grew on the m-7-h FC medium, but were unable to produce sufficient acid from lactose and mannitol during the 7- to 7.25-h incubation to lower the pH and turn the pH indicator yellow.

Mean unverified FC counts from the Lake Michigan field study were grouped according to the number of FC colonies per membrane filter for the standard 24-h m-FC procedure as shown in Table 6. The mean FC count per 100 ml for both the 24 h m-FC test and the 7-h FC test was determined, and the mean ratio 7-h FC/24-h FC was also calculated for each category. The highest ratios recorded were in the ≤5-colonies-per-membrane category and in the 6- to 10-colonies-per-membrane category. Low membrane filter colony counts frequently resulted in large differences in the 7-h FC/24-h FC ratio due to the random distribution of a few FC in a large sample volume. For all samples that had FC membrane filter counts recorded as a "less-than" (<) value for the sample volume filtered, the less-than value was treated as a definite number to permit estimation of the 7-h FC/24-h FC ratio. For example, if the FC count on the 7-h

TABLE 4. *FC verification of yellow colonies picked from m-7-h FC medium*

Source	No. of samples	No. of colonies picked	No. of colonies verified	% Verification
Ohio River, all sampling points	32	1,474	1,454	98.6
Creeks and surface runoff	31	1,202	1,160	96.5
Lakes and ponds	29	941	904	96.1
Total	92	3,617	3,518	97.3

TABLE 5. *FC verification of nonyellow colonies picked from m-7-h FC medium*

Colony description	No. of colonies picked	No. of colonies verified	% Verification
Clear, colorless	153	103	67.3
Purple	107	73	68.2
Brown	12	1	8.3

TABLE 3. *Statistical comparison of the mean ratio of the m-7-h FC to m-FC FC counts for each sample source*

Source	No. of samples	Mean ratio m-7-h FC/m-FC	95% confidence limits of mean	Standard error of mean	Variance of mean
Rivers and creeks					
Ohio River, 4 Seasons	4	0.89	0.81–0.97	0.04	0.01
Ohio River, Schmidt Field	7	0.82	0.60–1.04	0.11	0.08
Ohio River, Stadium	10	0.84	0.74–0.95	0.05	0.03
Ohio River, Riverside	10	1.08	0.84–1.32	0.12	0.15
Mill Creek	8	2.00	1.16–2.84	0.42	1.41
Duck Creek	8	0.95	0.86–1.04	0.05	0.02
Sycamore Creek	10	1.12	1.02–1.23	0.05	0.03
Lakes and ponds					
Cowan Lake	3	1.13	0.87–1.38	0.13	0.05
Stonelick Lake	3	1.10	0.84–1.35	0.13	0.05
Devou Park Lake	4	0.55	0.24–0.85	0.15	0.09
Winton Woods	5	0.90	0.64–1.16	0.13	0.08
Sharon Woods	6	1.04	0.71–1.37	0.17	0.16
Tupper Pond	3	0.85	0.68–1.03	0.09	0.02
Walton Reservoir	5	1.46	0.65–2.28	0.41	0.84

TABLE 6. *Lake Michigan field study results comparing the 7-h FC test with the 24-h FC test*[a]

Membrane colony count[b]	No. of samples	Mean 24-h FC/100 ml	Mean 7-h FC/100 ml	Mean ratio 7-h FC/24-h FC
≤5	719	8	13	1.88[c]
6–10	186	32	42	1.25
11–19	155	73	76	1.03
20–60	155	180	180	0.96
61–80	20	350	290	0.86
>80	55	1,000	750	0.89

[a] Data courtesy of Evanston-North Shore Health Department, Evanston, Ill.

[b] All data reported in this table based upon unverified FC counts.

[c] Mean of all sample ratios in colony count group.

FC membrane was 0/10 ml, the FC count was recorded as <10/100 ml [0/10 ml = <1/10 ml; 10(<1/10) = <10/100 ml]; therefore, a value of 10/100 ml was used to calculate the 7-h FC/24-h FC ratio.

The mean 7-h FC/24-h FC was highest for the ≤5 FC membrane colony count category and decreased in each subsequent category, reaching a low of 0.86 in the 61 to 80 membrane colony count category. The mean ratio was very close to 1.0 (0.96) for the 20 to 60 FC membrane colony count category, which is the countable range recommended by *Standard Methods* (1) for the 24-h FC procedure.

Data from the Lake Michigan field study are summarized in Table 7. Only the data from sample volumes that gave 20 to 60 FC colonies per membrane filter were included. Variations in the mean ratio (m-7-h FC/24-h FC) did not correlate with season. The mean ratio 7-h FC/24-h FC varied from a low of 0.76 to a high of 1.11; the mean ratio for all samples was 0.95. Thus, the 7-h FC test provided FC results equivalent to those obtained by the standard 24-h FC test.

Results from field tests in which the 7-h FC test was used to enumerate FC in unchlorinated treated sewage effluents are shown in Table 8. The data show that the verified FC count from the 7-h FC test averaged about 2 to 5 times higher than the 24-h FC count. The percent verification of yellow FC colonies picked from the m-7-h FC plates compared very favorably with the percent verification of blue FC colonies picked from the standard 24-h m-FC plates. Higher FC counts obtained from the 7-h FC test indicate that stressed FC were able to survive and multiply when incubated at 41.5°C, whereas 44.5°C incubation apparently resulted in the death of some of the stressed FC. The high verification percentages for the 7-h FC medium tend to support this explanation.

TABLE 7. *Summary of Lake Michigan field study*[a] *data for membrane filter FC colony counts in the 20 to 60 colony range*[b]

Year/month	No. of samples	Mean 24-h FC/100 ml	Mean 7-h FC/100 ml	Mean ratio 7-h FC/24-h FC
1975				
June	7	220	270	0.87[c]
July	30	180	140	0.76
August	37	270	290	1.11
1976				
June	13	66	47	0.84
July	42	150	160	1.00
August	22	140	130	0.94
September	4	150	130	0.90
Total	155	180	180	0.95

[a] Data courtesy of Evanston-North Shore Health Department, Evanston, Ill.

[b] All average values calculated from only those samples that had 20 to 60 FC colonies per membrane filter in the 24-h FC test and the corresponding 7-h FC colony counts.

[c] Mean ratio calculations based on the number of samples analyzed during the month, not the mean 24-h FC counts and mean 7-h FC counts shown in the table.

The data shown in Table 9 compare FC results from laboratory-prepared m-7-h FC medium versus results from two commercially prepared trial batches of m-7-h FC medium. Examination of the mean 7-h FC/24-h FC ratios for samples from four different water sources showed that both commercially prepared batches of the m-7-h FC medium performed somewhat less satisfactorily than the laboratory-prepared medium. Compared with the standard 24-h FC test, the ranking of the media from best to poorest results was laboratory m-7-h FC > Difco m-7-h FC > BBL m-7-h FC.

Table 10 summarizes unverified FC data from Lake Michigan water samples examined by using the commercial trial batches of m-7-h FC medium. Because of the number of daily beach-monitoring samples, verification of all suspected FC colonies was not possible, although a limited number of colonies was picked at random for verification. Examination of the data in Table 10 shows that the results from the Lake Michigan water samples with Difco m-7-h FC medium and BBL 7-h FC medium were similar for most membrane colony count categories; thus, the differences observed did not appear to be significant. For this particular sample source, both media performed satisfactorily. The data in Tables 9 and 10 appear to conflict in that the

TABLE 8. *Comparison of verified 24-h FC and 7-h FC results from examination of unchlorinated secondary treated wastewaters*[a]

Source/date	Verified FC/100 ml[b]		Mean ratio 7-h FC/ 24-h FC	% Verification	
	24-h FC	7-h FC		24-h FC	7-h FC
Piscataway Treatment Plant					
4 February 1976	400	3,400	8.50	67	48
5 February 1976	400	2,600	6.50	67	87
7 February 1976	7,200	7,800	1.08	100	98
16 February 1976	6,600	6,600	1.82	100	94
17 February 1976	19,000	50,000	2.63	90	100
18 February 1976	2,600	10,000	3.85	87	85
23 February 1976	2,600	2,800	1.08	100	82
24 February 1976	3,000	7,400	2.47	100	95
25 February 1976	4,400	7,400	1.68	100	95
1 March 1976	400	10,000	27.50	100	100
2 March 1976	4,600	24,000	5.22	100	100
3 March 1976	2,800	16,000	5.71	100	100
9 March 1976	7,200	11,000	1.53	95	100
10 March 1976	5,600	6,200	1.11	82	100
Avg	4,800	12,000	5.05	92	92
Parkway Treatment Plant					
22 March 1976	3,000	9,000	3.00	100	90
24 March 1976	5,100	14,000	2.74	85	75
29 March 1976	5,400	8,700	1.61	100	85
30 March 1976	3,000	6,100	2.03	100	93
31 March 1976	5,200	13,000	2.50	100	85
5 April 1976	7,300	25,000	3.42	94	85
6 April 1976	4,200	25,000	5.95	91	80
7 April 1976	6,600	17,000	2.57	94	80
28 April 1976	23,000	38,000	1.65	100	95
3 May 1976	2,500	2,700	1.08	100	50
5 May 1976	23,000	38,000	1.65	98	100
10 May 1976	4,000	5,000	1.25	88	77
10 May 1976	6,700	23,000	3.43	95	89
17 May 1976	40,000	39,000	0.98	91	100
Avg	9,900	18,000	2.56	95	85
Landover Treatment Plant					
20 September 1976	20,000	24,000	1.20	100	83
21 September 1976	10,000	24,000	2.40	77	95
22 September 1976	5,000	3,000	0.60	100	100
27 September 1976	2,500	2,000	0.80	100	100
28 September 1976	3,300	7,600	2.30	100	95
29 September 1976	1,000	6,300	6.30	100	95
5 October 1976	20,000	31,000	1.55	90	100
12 October 1976	32,000	38,000	1.19	90	90
19 October 1976	1,000	3,700	3.70	100	91
20 October 1976	5,000	6,000	1.20	93	100
25 October 1976	24,000	18,000	0.75	95	95
26 October 1976	7,000	10,000	1.43	93	95
Avg	9,100	14,000	1.95	95	95

[a] Data provided by Raul J. Celorio, Washington Suburban Sanitary Commission, Hyattsville, Md.

performance of each commercially prepared m-7-h FC medium varied to some extent with the type of water sample, except for the Lake Michigan samples. Both Difco m-7-h FC and BBL m-7-h FC media performed equally well on the Lake Michigan samples (Table 10), yet the performance of the BBL m-7-h FC medium was much poorer than the Difco m-7-h FC medium on other water samples (Table 9). The reason for this difference is unclear, although the m-7-h FC medium is only very lightly buffered and pH, turbidity, and dissolved solids of the water sample may have resulted in differences in the amount of acid production necessary to shift the pH indicator into the yellow range.

A total of 277 yellow, pale yellow, clear, and purple colonies were picked from m-7-h FC plates, streaked for pure culture, and characterized and identified by using the API-20E System (Analytab Products, Division of Ayerst Laboratories, Inc.) (Table 11). *Escherichia coli* comprised 59.9% of the cultures, followed by: *Klebsiella pneumoniae*, 31.8%; *Enterobacter* sp., 6.5%; *Pseudomonas* sp., 0.7%; and *Citrobacter*

TABLE 9. *Summary of results comparing verified FC counts on laboratory-prepared m-7-h FC medium versus commercially prepared m-7-h FC media*

Source	No. samples	24-h FC/100 ml	7-h FC/100 ml			Mean ratio		
			Lab	Difco	BBL	Lab 7 h/24 h	Difco 7 h/24 h	BBL 7 h/24 h
Mill Creek								
a	8	2,900	7,000 (92)[a]	6,900 (88)		2.01	1.95	
b	8	80,000	66,000 (99)		51,000 (99)	0.80		0.64
Ohio River, Riverside								
a	10	8,700	8,000 (99)	9,300 (98)		1.07	1.14	
b	10	6,200	5,300 (98)		3,900 (97)	0.90		0.68
Sycamore Creek								
a	10	240	240 (99)	240 (99.6)		1.0	1.0	
b	10	29,000	23,000 (95)		15,000 (93)	0.80		0.58
Walton Reservoir								
a	5	90	110 (95)	110 (93)	(ND)	1.22	1.22	

[a] Numbers in parentheses are percent fecal coliform verification. ND, Not done.

TABLE 10. *Comparison of unverified FC counts on Lake Michigan water with Difco m-7-h FC and BBL m-7-h FC media*

MF colony count range	No. of samples	Mean 24-h FC/100 ml	Mean 7-h FC/100 ml (Difco)	Mean 7-h FC/100 ml (BBL)	Mean ratio 7-h FC/24-h FC (Difco)	Mean ratio 7-h FC/24-h FC (BBL)
≤5	180	3	3	4	1.40	1.57
6–10	67	8	6	8	0.80	1.02
11–20	53	16	8	17	0.90	1.07
21–60	81	37	39	43	1.00	1.12
61–80	20	72	59	69	0.76	0.98
81–110	18	103	113	75	0.88	0.71

fruendii and *Acinetobacter calcoaceticus*, 0.4% each. The proportion of either clear or purple colonies that confirmed as FC positive *E. coli* of *K. pneumoniae* varied with the sample source (data not shown). For example, most clear (69%) and purple (82%) colonies picked from Ohio River samples were identified as *K. pneumoniae*, but for Sycamore Creek most clear (78%) and purple (63%) colonies were identified as *E. coli*. Thus, the sample source and its characteristic pollution load apparently influence which coliform group organisms will be predominant in the clear and purple colony groups.

Initially, the large number of isolates from each colony color group that were verified as FC was disconcerting. However, the fact that the yellow (all shades) FC colony counts on the m-7-h FC medium were generally equivalent to the blue FC colony counts on the 24-h m-FC medium indicates that roughly the same populations of FC were counted. On the m-7-h FC medium, however, additional FC were recovered, probably due to the 41.5 ± 0.5°C incubation temperature. The FC strains that formed clear or purple colonies apparently were injured or physiologically compromised organisms that probably could not survive exposure to 44.5 ± 0.2°C but were able to repair and grow at 41.5 ± 0.5°C even though insufficient acidity was produced to shift the pH indicator into the yellow range. Thus, the m-7-h FC medium offers a selective means of recovering many of the stressed FC that may be present in water.

DISCUSSION

During early development of the medium, fade out of the yellow color of FC colonies was noted beginning about 15 min after the m-7-h FC plates were removed from the 41.5°C water bath. Incorporation of 0.5% mannitol in the medium eliminated fade away of the yellow color. Apparently, the additional acid produced during mannitol fermentation was sufficient to maintain the pH in the yellow color range of the bromocresol purple even after the plates were placed at room temperature before counting. Total substitution of mannitol in place of lactose was not satisfactory; yellow colony color development was best only when both lactose and mannitol were present. Diauxic growth was not noted, and, due to the length of incubation, appeared not to be of concern in this procedure.

TABLE 11. *Identification of colonies picked from m-7-h FC media*

Colony color	No. picked		Identification by no.	API 20E System organism
	Total	EC+		
Yellow	61	59	4	*K. pneumoniae*
			53	*E. coli* (1 FC−)
			2	*Pseudomonas* sp. (1 FC+)
			2	*E. aerogenes* (1 FC+)
Pale yellow	15	11	10	*E. coli* (1 FC−)
			4	*E. cloacae* (1 FC+)
			1	*C. freundii*
Clear	44	39	17	*K. pneumoniae*
			22	*E. coli*
			4	*E. aerogenes* (all FC−)
			1	*E. cloacae* (FC−)
Purple	157	145	67	*K. pneumoniae* (5 FC−)
			81	*E. coli*
			4	*E. aerogenes* (3 FC−)
			3	*E. cloacae* (2 FC−)
			1	*E. hafniae* (FC−)
			1	*A. calcoaceticus* (FC−)

Incorporation of two fermentable substrates offers stressed organisms the option of two metabolic pathways and facilitates more rapid repair and growth of the injured organisms.

Mannitol is used fermentatively by most members of the *Enterobacteriaceae*, including 96% of the genus *Escherichia* (6). Fennell (7) reported the use of mannitol in a single-tube confirmatory test for *E. coli* and found that because late lactose-fermenting and lactose-negative *E. coli* were detected, a better presumptive *E. coli* count at 44°C was obtained. Pugsley et al. (13) found that the substitution of mannitol for lactose did not result in an increase in false-negative or false-positive reactions in the 44°C confirmatory test for *E. coli*. Therefore, the use of mannitol as a fermentable substrate in the m-7-h FC medium is supported by the experience of other investigators as well as our experimental results.

Incubation at 41.5°C is a compromise between greater selectivity for FC when 44.5°C incubation is used and enriched growth of all other enteric organisms. The result is that there apparently is optimum differential growth of FC that includes increased recovery of stressed FC. Thus, the reduction in incubation temperature from 44.5°C as used in the 24-h FC procedure to 41.5°C for the 7-h FC test undoubtedly contributed directly to the large number of nonyellow colonies that were confirmed as FC. These organisms are believed to be stressed FC that would fail to survive if incubated at 44.5°C on m-FC agar. Reports by other investigators have shown that significant numbers of stressed cells exist in populations of bacteria exposed to aquatic environments or foods (2, 5, 10, 16). Modified culture methods are required for recovery and enumeration of sublethally stressed organisms (3, 4, 8, 9, 11, 12, 14, 15). On the basis of such evidence, it is reasonable to conclude that the 41.5°C incubation temperature resulted in less additional stress to injured FC than did 44.5°C incubation. Thus, more FC were able to repair metabolic damage and grow. Given additional time to grow, they would have produced sufficient acid to shift the indicator system into the yellow color range. When 7-h FC plates were left at room temperature overnight, the plates turned partially or totally yellow within 12 to 24 h.

The fact that the FC counts from the 7-h FC test and the 24-h FC test generally agreed well indicates that, regardless of the number of nonyellow colonies that may be confirmed as FC coliforms, the 7-h FC test measured a FC population comparable to that recovered in the 24-h test. Thus, the 7-h FC test makes it possible to obtain FC estimates within an 8-h period of time. Confirmation of the yellow colonies as FC requires an additional 48 h to carry the isolates through lauryl tryptose broth and EC broth.

When applied as a monitoring procedure, the 7-h FC test should initially be run in parallel with the 24-h FC test to establish that it provides equivalent results. The user should confirm not only the yellow FC colonies, but also some of the purple and clear colonies to determine the predominant FC organism in each colony type.

The 7-h FC test provides rapid, quantitative FC results and can be used for the emergency examination of potable water in the event of a line break, cross-connection, or other occurrence in which fecal or sewage contamination occurred. It is particularly useful for rapid detection of recreational water quality changes related to storm water runoff, sanitary waste spills or bypasses, and for effluent monitoring for treatment malfunction.

ACKNOWLEDGMENTS

We are grateful for the able assistance of Harriet Kennedy and her staff at the Evanston-Northshore Health Department,

Evanston, Ill., who provided the Lake Michigan field study data and for the assistance of Raul J. Celorio and staff at the Washington Suburban Sanitation Commission, Hyattsville, Md., who provided the unchlorinated wastewater data.

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