

APPENDIX B

FECAL COLIFORM MPN AND MEMBRANE FILTER TESTING PROCEDURES

According to the Standard Methods (1989), elevated-temperature tests for the separation of organisms of the coliform group into those of possible fecal origin and those derived from nonfecal sources are available. These tests can be performed by either multiple-tube procedures (MPN) or by membrane filter methods.

B.1 MPN Tests

There are two kinds of media that can be used to perform MPN tests. The first is to use EC medium and is applicable to investigations of stream pollution, raw water sources, wastewater treatment systems, bathing waters, seawaters, and general water-quality monitoring. However, this test should not be used for direct isolation of coliform from water because prior enrichment is required in a presumptive medium for optimum recovery of fecal coliform. The second is to use A-1 medium and is applicable to seawater and treated wastewater. For analyzing water samples collected by TDH for shellfish monitoring purpose, the A-1 medium is used. The following is a detailed description of the fecal coliform MPN procedures using A-1 medium.

The procedures begin with the preparation of the A-1 broth which includes the following ingredients:

Lactose	5.0 g
Tryptose	20.0 g
Sodium chloride, NaCl	5.0 g
Salicin	0.5 g
Polyethylene glycol p-isooctylphenyl ether	1.0 mL
Distilled water	1.0 L

The broth should be heated to dissolve solid ingredients, added polyethylene glycol p-isooctylphenyl ether, and adjusted to pH 6.9 ± 0.1 . Before sterilization dispense sufficient medium to cover the inverted vial in fermentation tubes at least partially after sterilization. Close with metal or heat-resistant plastic caps. Sterilize by autoclaving at 121°C for 10 minutes.

Arrange fermentation tubes in rows of five tubes each in a test tube rack. The number of five tube rows and the sample volumes selected depend upon the quality and character of the water to be examined. For potable water use five 10-mL portions or ten 10-mL portions; for nonpotable water use five tubes per dilution. Shake sample and dilutions vigorously about 25 times. Inoculate each tube of the set of five with replicate sample volumes. Mix test portions in the medium by gentle agitation. Incubate for 3 hours at $35 \pm 0.5^\circ\text{C}$. Transfer tubes to a water bath at $44.5 \pm 0.2^\circ\text{C}$ and incubate for an additional 21 ± 2 hours.

Gas production in an A-1 broth culture within 24 hours or less is considered a positive fecal coliform reaction. Failure to produce gas (growth sometimes occurs) constitutes a negative reaction indicating a source other than the intestinal tract of warm-blooded animals. MPN value can be calculated from the number of positive A-1 broth tubes as follows (Standard Methods, 1989):

$$\text{MPN} / 100 \text{ mL} = \text{MPN Index} * \frac{10}{\text{largest volume tested}}$$

where MPN Index can be obtained from the following tables:

a. When five 10-mL portions are used:

No. of Tubes Giving Positive Reaction Out of 5 of 10 mL Each	MPN Index / 100 mL	95% Confidence Limits	
		Lower	Upper
0	<2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.0	3.3	52.9
5	> 16.0	8.0	Infinite

b. When ten 10-mL portions are used:

No. of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index / 100 mL	95% Confidence Limits	
		Lower	Upper
0	<1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

B.2 Fecal Coliform Membrane Filter Procedure

The membrane filter (MF) procedure uses an enriched lactose medium and incubation temperature of $44.5 \pm 0.2^\circ\text{C}$ for selectivity and is said to give 93% accuracy in differentiating between coliform found in the feces of warm-blooded animals and those from other environmental sources (Standard Methods, 1989). The test is used by TWC and can be described as follows.

The ingredients of M-FC medium for membrane filter test are:

Tryptose or biosate	10.0 g
Proteose peptone No. 3 or polypeptone	5.0 g
Yeast extract	3.0 g
Sodium chloride, NaCl	5.0 g
Lactose	12.5 g
Bile salts mixture or bile salts No. 3	1.5 g
Aniline blue	0.1 g
Distilled water	1.0 L

Rehydrate in distilled water containing 10 mL 1% rosolic acid in 0.2N NaOH. Heat to near boiling, promptly remove from heat, and cool to below 50°C. Do not sterilize by autoclaving. Dispense 5-to 7- mL quantities to 50- * 12-mm petri plates and let solidify if agar is used. Final pH should be 7.4. Store finished medium at 2 to 10°C.

Volume of water sample to be examined is selected in accordance with the following table. Only sample volumes that will yield counts between 20 and 60 fecal coliform colonies per membrane should be used.

Water Source	Volume to be Filtered (mL)						
	100	50	10	1	0.1	0.01	0.001
Lakes, reservoirs	X	X					
Wells, spring	X	X					
Water supply intake		X	X	X			
Natural bathing waters		X	X	X			
Sewage treatment plant, secondary effluent			X	X	X		
Farm ponds, rivers				X	X	X	
Stormwater runoff				X	X	X	
Raw municipal sewage					X	X	X
Feedlot runoff					X	X	X

Using sterile forceps, place a sterile membrane filter over porous plate of receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place, rinse funnel by filtering three 20- to 30-mL portions of sterile dilution water. Upon completion of final rinse and the filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place it on M-FC medium with a rolling motion to avoid entrapment of air.

Place a sterile absorbent pad in each culture dish and pipet approximately 2 mL M-FC medium to saturate pad. Place prepared filter on medium-impregnated pad. Place prepared cultures in waterproof plastic bags or seal petri dishes, submerge in water bath, and incubate for 24 ± 2 hours at $44.5 \pm 0.2^\circ\text{C}$. Anchor dishes below water surface to maintain critical temperature requirements. All prepared cultures should be placed in the water bath within 30 minutes after filtration.

Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Nonfecal coliform colonies are gray to cream-colored. Count colonies with a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope or other optical device. Compute the fecal coliform density from the sample quantities that produced membrane filter counts within the desired range of 20 to 60 fecal coliform colonies. The density of fecal coliform can be computed by

$$\text{coliform colonies/100 mL} = \frac{\text{coliform colonies counted}}{\text{mL sample filtered}} * 100$$