9222 A. Introduction

The membrane filter (MF) technique is highly reproducible, can be used to test relatively large sample volumes, and usually yields numerical results more rapidly than the multiple-tube fermentation procedure. The MF technique is extremely useful in monitoring drinking water and a variety of natural waters. However, the MF technique has limitations, particularly when testing waters with high turbidity or large numbers of noncoliform (background) bacteria. When the MF technique has not been used previously, it is desirable to conduct parallel tests with the method the laboratory is using currently to demonstrate applicability and comparability.

1. Definition

As related to the MF technique, the coliform group is defined as those facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that develop red colonies with a metallic (golden) sheen within 24 h at 35°C on an Endo-type medium containing lactose. Some members of the total coliform group may produce dark red, mucoid, or nucleated colonies without a metallic sheen. When verified these are classified as atypical coliform colonies. When purified cultures of coliform bacteria are tested, they produce negative cytochrome oxidase and positive β-galactosidase test reactions.†#(2) Generally, pink (non-mucoid), blue, white, or colorless colonies lacking sheen are considered noncoliforms by this technique.

2. Applications

Turbidity caused by the presence of algae, particulates, or other interfering material may not permit testing of a sample volume sufficient to yield significant results. Low coliform estimates may be caused by the presence of high numbers of noncoliforms or of toxic substances. The MF technique is applicable to the examination of saline waters, but not wastewaters that have received only primary treatment followed by chlorination because of turbidity in high volume samples or wastewaters containing toxic metals or toxic organic compounds such as phenols. For the detection of stressed total coliforms in treated drinking water and chlorinated secondary or tertiary wastewater effluents, use a method designed for stressed organism recovery (see Section 9212B.1). A modified MF technique for fecal coliforms (Section 9212) in chlorinated wastewater may be used if parallel testing over a 3-month period with the multiple-tube fermentation technique shows comparability for each site-specific type of sample.

The standard volume to be filtered for drinking water samples is 100 mL. This may be distributed among multiple membranes if necessary. However, for special monitoring purposes, such as troubleshooting water quality problems or identification of coliform breakthrough in low concentrations from treatment barriers, it may be desirable to test 1-L samples. If particulates prevent filtering a 1-L sample through a single filter, divide sample into four portions of 250 mL for analysis. Total the coliform counts on each membrane to report the number of coliforms per liter. Smaller sample volumes will be necessary for source or recreational waters and wastewater

effluents that have much higher coliform densities.

Statistical comparisons of results obtained by the multiple-tube method and the MF technique show that the MF is more precise (compare Table 9221:II and Table 9221:III with Table 9222:II). Data from each test yield approximately the same water quality information, although numerical results are not identical.

3. Bibliography

CLARK, H.F., E.E. GELDREICH, H.L. JETER & P.W. KABLER. 1951. The membrane filter in sanitary bacteriology. *Pub. Health Rep.* 66:951. KABLER, P.W. 1954. Water examinations by membrane filter and MPN procedures. *Amer. J. Pub. Health* 44:379. THOMAS, H.A. & R.L. WOODWARD. 1956. Use of molecular filter membranes for water potability control. *J. Amer. Water Works Assoc.* 48: 1391. MCCARTHY, J.A., J.E. DELANEY & R.J. GRASSO. 1961. Measuring coliforms in water. *Water Sewage Works* 108:238. LIN, S. 1973. Evaluation of coliform test for chlorinated secondary effluents. *J. Water Pollut. Control Fed.* 45:498. MANDEL, J. & L.F. NANNI. 1978. Measurement evaluation. *In* S.L. Inhorn, ed. Quality Assurance Practices for Health Laboratories, p. 209. American Public Health Assoc., Washington, D.C.

9222 D. Fecal Coliform Membrane Filter Procedure

Fecal coliform bacterial densities may be determined either by the multiple-tube procedure or by the MF technique. See Section 9225 for differentiation of *Escherichia coli*, the predominant fecal coliform. If the MF procedure is used for chlorinated effluents, demonstrate that it gives comparable information to that obtainable by the multiple-tube test before accepting it as an alternative. The fecal coliform MF procedure uses an enriched lactose medium and incubation temperature of 44.5 ± 0.2°C for selectivity. Because incubation temperature is critical, submerge waterproofed (plastic bag enclosures) MF cultures in a water bath for incubation at the elevated temperature or use an appropriate solid heat sink incubator or other incubator that is documented to hold the 44.5°C temperature within 0.2°C throughout the chamber, over a 24-h period. Areas of application for the fecal coliform method in general are stated in the introduction to the multiple-tube fecal coliform procedures, Section 9221E.

1. Materials and Culture Medium

a. M-FC medium: The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. Commercially prepared media in liquid form (sterile ampule or other) also may be used if known to give equivalent results. See Section 9020 for quality control specifications.

M-FC medium:

Tryptose or biosate 10.0g

Proteose peptone No. 3 or polypeptone 5.0g

Yeast Extract 3.0g

Sodium Chloride, NaCl 5.0g

Lactose 12.5g

Bile Salts No. 3 or bile salts mixture 1.5g

Aniline blue 0.1g

Agar (optional) 15g

Reagent-grade water 1L

Rehydrate product in 1 L water containing 10 mL 1% rosolic acid in 0.2*N* NaOH.*#(3) Heat to near boiling, promptly remove from heat, and cool to below 50°C. Do not sterilize by autoclaving. If agar is used, dispense 5- to 7-mL quantities to 50- × 12-mm petri plates and let solidify. Final pH should be 7.4 ± 0.2. Refrigerate finished medium, preferably in sealed plastic bags or other containers to reduce moisture loss, and discard unused broth after 96 h or unused agar after 2 weeks.

Test each medium lot against a previously acceptable lot for satisfactory performance as described in Section 9020B, by making dilutions of a culture of *E. coli* (Section 9020) and filtering appropriate volumes to give 20 to 60 colonies per filter. With each new lot of medium verify 10 or more colonies obtained from several natural samples, to establish the absence of false positives. For most samples M-FC medium may be used without the 1% rosolic acid addition, provided there is no interference with background growth. Such interference may be expected in stormwater samples collected during the first runoff (initial flushing) after a long dry period.

Before use, test each batch of laboratory-prepared MF medium for performance with positive and negative culture controls. Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30 mL of dilution or rinse water through filter. If controls indicate contamination, reject all data from affected samples and request resample.

b. Culture dishes: Tight-fitting plastic dishes are preferred because the membrane filter cultures are submerged in a water bath during incubation. Place fecal coliform cultures in plastic bags or seal individual dishes with waterproof (freezer) tape to prevent leakage during submersion. Specifications for plastic culture dishes are given in Section 9222B.1*e*.

c. Incubator: The specificity of the fecal coliform test is related directly to the incubation temperature. Static air incubation may be a problem in some types of incubators because of potential heat layering within the chamber, slower heat transfer from air to the medium, and the slow recovery of temperature each time the incubator is opened during daily operations. To meet the need for greater temperature control use a water bath, a heat-sink incubator, or a properly designed and constructed incubator shown to give equivalent results. A temperature tolerance of

44.5 ± 0.2°C can be obtained with most types of water baths that also are equipped with a gable top for the reduction of water and heat losses.

2. Procedure

a. Selection of sample size: Select volume of water sample to be examined in accordance with the information in Table 9222:III. Use sample volumes that will yield counts between 20 and 60 fecal coliform colonies per membrane.

When the bacterial density of the sample is unknown, filter several volumes or dilutions to achieve a countable density. Estimate volume and/or dilution expected to yield a countable membrane and select two additional quantities representing one-tenth and ten times this volume, respectively.

b. Filtration of sample: Follow the same procedure and precautions as prescribed under Section 9222B.5*b* above.

c. Preparation of culture dish: Place a sterile absorbent pad in each culture dish and pipet at least 2.0 mL M-FC medium, prepared as directed above, to saturate pad. Carefully remove any excess liquid from culture dish by decanting the plate. Aseptically, place prepared filter on medium-impregnated pad as described in Section 9222B above.

As a substrate substitution for the nutrient-saturated absorbent pad, add 1.5% agar to M-FC broth as described in Section 9222B above.

d. Incubation: Place prepared dishes in waterproof plastic bags or seal, invert, and submerge petri dishes in water bath, and incubate for 24 ± 2 h at 44.5 ± 0.2°C. Anchor dishes below water surface to maintain critical temperature requirements. Place all prepared cultures in the water bath within 30 min after filtration. Alternatively, use an appropriate, accurate solid heat sink or equivalent incubator.

e. Counting: Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Nonfecal coliform colonies are gray to cream-colored. Normally, few nonfecal coliform colonies will be observed on M-FC medium because of selective action of the elevated temperature and addition of rosolic acid salt reagent. Count colonies with a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope or other optical device.

f. Verification: Verify typical blue colonies and any atypical grey to green colonies as described in Section 9020 for fecal coliform analysis. Simultaneous inoculation at both temperatures is acceptable.

3. Calculation of Fecal Coliform Density

a. General: Compute the density from the sample quantities that produced MF counts within the desired range of 20 to 60 fecal coliform colonies. This colony density range is more restrictive than the 20 to 80 total coliform range because of larger colony size on M-FC medium. Calculate fecal coliform density as directed in Section 9222B.6 above. Record densities as fecal coliforms per l00 mL.

b. Sediment and biosolid samples: For total solid (dry weight basis) see Section 2540G. Calculate fecal coliforms per gram dry weight for biosolid analysis as follows: where dilution and % dry solids are expressed in decimal form.

Fecal coliforms per gram dry weight = colonies counted/ (dilution chosen) x (% dry solids)

Example 1: There were 22 colonies observed on the 1:10 000 dilution plate of a biosolid with 4% dry solids.

22/(0.0001) X (0.04) = 5.5 x 10^6 fecal coliform/g dry weight

If no filter has a coliform count falling in the ideal range (20 to 60), total the coliform counts on all countable filters and report as fecal coliforms per gram dry weight.

Example 2: There were 18 colonies observed on the 1:10 000 dilution plate and 2 colonies observed on the 1:100 000 dilution plate of a biosolid sample with 4% dry solids.

(18+2) /(0.0001 + 0.00001) (0.04) = 4.5 x 10^6

To compute a geometric mean of samples, convert coliform densities of each sample to log10 values. Determine the geometric mean for the given number of samples (usually seven) by averaging the log10 values of the coliform densities and taking the antilog of that value.

4. Bibliography

GELDREICH, E.E., H.F. CLARK, C.B. HUFF & L.C. BEST. 1965. Fecal-coliform-organism medium for the membrane filter technique. *J. Amer. Water Works Assoc*. 57:208.

ROSE, R.E., E.E. GELDREICH & W. LITSKY. 1975. Improved membrane filter method for fecal coliform analysis. *Appl. Microbiol*. 29:532. LIN, S.D. 1976. Membrane filter method for recovery of fecal coliforms in chlorinated sewage effluents. *Appl. Environ. Microbiol*. 32:547. PRESSWOOD, W.G. & D.K. STRONG. 1978. Modification of M-FC medium by eliminating rosolic acid. *Appl. Environ. Microbiol*. 36:90. GREEN, B.L., W. LITSKY & K.J. SLADEK. 1980. Evaluation of membrane filter methods for enumeration of faecal coliforms from marine waters. *Mar. Environ. Res*. 67:267. SARTORY, D.P. 1980. Membrane filtration faecal coliform determinations with unmodified and modified M-FC medium. *Water SA* 6:113.

GRABOW, W.O.K., C.A. HILNER & P. COUBROUGH. 1981. Evaluation of standard and modified M-FC, MacConkey, and Teepol media for membrane filter counting of fecal coliform in water. *Appl. Environ. Microbiol*. 42:192.

RYCHERT, R.C. & G.R. STEPHENSON. 1981. Atypical *Escherichia coli* in streams. *Appl. Environ. Microbiol*. 41:1276. PAGEL, J.E., A.A. QURESHI, D.M. YOUNG & L.T. VLASSOFF. 1982. Comparison of four membrane filter methods for fecal coliform enumeration. *Appl. Environ. Microbiol*. 43:787.

ENVIRONMENTAL PROTECTION AGENCY. 1992. Environmental Regulations and Technology. Control of Pathogens and Vector Attraction in Sewage Sludge. EPA-626/R-92-013, Washington, D.C.

ENVIRONMENTAL PROTECTION AGENCY. 1993. Standards for the Use or Disposal of Sewage Sludge: Final Rule. 40 CFR Part 257; *Federal Register* 58:9248, Feb. 19, 1993.