Coliforms—Fecal

DOC316.53.001209

USEPA Membrane Filtration Method **Method 8074[1]**

m-FC and m-FC/RA

Scope and Application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA approved 9222 D.

Introduction

The Membrane Filtration (MF) method is a fast way to estimate bacterial populations in water. The MF method is especially useful when evaluating large sample volumes or performing many coliform tests daily.

Method

In the initial step, an appropriate sample volume passes through a membrane filter with a pore size small enough (0.45 micron) to retain the bacteria present. The filter is placed on an agar plate prepared with a culture medium that is selective for coliform growth. The petri dish is incubated, upside down, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown are identified and counted using a low-power microscope.

PourRite™ Ampules contain prepared selective media. This eliminates the measuring, mixing, and autoclaving needed when preparing dehydrated media. The ampules are designed with a large, unrestrictive opening that allows media to pour out easily. Each ampule contains enough medium for one test.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite™ ampules, allow the media to warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

Nonpotable waters procedures

Wastewater, river, bathing, and other nonpotable waters usually are tested for fecal coliforms. In testing for fecal coliforms, a special medium and an elevated incubation temperature inhibit growth of nonfecal coliforms. Fecal coliforms growing on the membrane form an acid that reacts with an aniline dye in the medium, producing a blue color.

Use m-FC Broth with Rosolic Acid to increase specificity when high levels of non-coliform bacteria may be present, unless all the organisms in the sample are stressed or injured.

Confirmation of fecal coliforms (m-FC or m-FC/RA), method 8074

1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the petri dish lid.

Do not touch the pad or the inside of the petri dish.

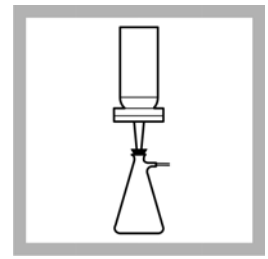
To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

Petri dishes with pads are available.

2. Invert an m-FC Broth PourRite Ampule 2 to 3 times to mix the broth. Use the ampule breaker to open an ampule. Carefully pour the contents evenly onto the absorbent pad. Replace the petri dish lid.

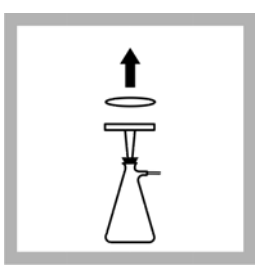
Use m-FC Broth with Rosolic Acid to increase specificity when high levels of non-coliform bacteria may be present, unless the organisms are stressed or injured.

3. Set up the Membrane Filter Assembly. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.

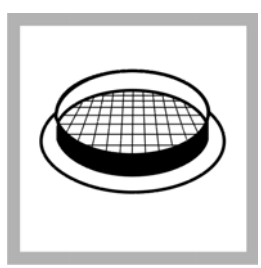


4. Prepare the necessary dilutions to obtain the proper sample size. Invert the sample for 30 seconds to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Repeat rinsing step, two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.



5. Turn off the vacuum and lift off the funnel top. Use sterile forceps to transfer the membrane filter to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure the entire filter touches the pad. Replace the petri dish lid.

7. Invert the petri dish and incubate at 44.5 ± 0.2 °C for 24 ± 2 hours.

To eliminate environmental Klebsiella from the fecal coliform population elevate the temperature to 45.0 ± 0.2 °C.

Alternatively, a water bath with rack may be used for incubation by placing the petri dishes into a sealed bag.

8. After incubating, count the blue colonies using a 10 to 15X microscope.

Confirmation of fecal coliforms (m-FC or m-FC/RA), method 8074 (continued)

9. Record the results of the test. See *Interpreting and reporting results*.

To verify results, follow *Verifying fecal coliforms, method 8074*

Confirmation of total coliforms (Lauryl Tryptose and Brilliant Green Bile)

For potable water samples, confirm typical colonies to ensure they are coliforms. (Confirm sheen colonies, up to a maximum of five.) Inoculate parallel tubes of Lauryl Tryptose (LT) Single Strength (SS) Broth and Brilliant Green Bile (BGB) Broth by transferring growth from each colony. Growth and gas production in both tubes verifies that the suspect organisms are coliforms. Most Probable Number (MPN) coliform tubes are ideal for this purpose.

Use the swabbing technique for fecal coliforms or *E. coli*:

- When determining only the presence or absence of total coliforms
- When inoculating EC or EC/MUG media

Inoculate in this order:

1. EC or EC/MUG

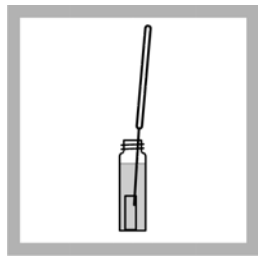
2. LT SS Broth

3. BGB

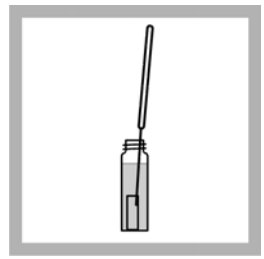
Confirmation of total coliforms (LT and BGB), method 8074

1. Sterilize an inoculating needle, or use a sterile, disposable inoculating needle.

To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.

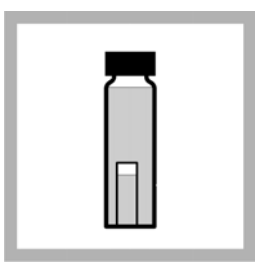


2. Touch the needle to the coliform (sheen) colony grown on m-Endo Broth. Transfer to a single-strength Lauryl Tryptose (LT) Broth tube.



3. Again touch the same coliform colony with the needle. Transfer to a Brilliant Green Bile (BGB) Broth tube.

4. Invert both tubes to eliminate any air bubbles trapped in the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial, then continue incubation.



5. After 24 ± 2 hours, check the inner vials for growth and gas bubbles. Growth (turbidity) and gas bubbles in both the LT and BGB Broth tubes verify that the colonies are coliforms. If one or both tubes do not show gas, continue incubating both tubes for an additional 24 hours

6. If no gas is present in the LT Broth tube after 48 hours, the colony is not a coliform and additional testing is unnecessary.

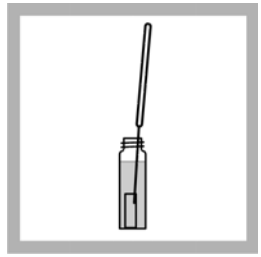
Record the results of the test. See *Interpreting and reporting results*

Confirm positive results. If growth and gas are produced in the LT Broth tube but not in the BGB Broth tube, inoculate another BGB tube from the gas-positive LT Broth tube. Incubate this BGB Broth tube and check for growth and gas after 24 hours and/or after 48 hours. If growth and gas are produced within 48 ± 3 hours, the colony is confirmed as coliform.

Verifying fecal coliforms, method 8074

1. Sterilize an inoculating needle, or use a sterile, disposable inoculating needle.

To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner flame. Let the needle cool before use.



2. Touch the needle to a typical blue colony and transfer to a Lauryl Tryptose (LT) Broth tube. Repeat steps 1 and 3 for each test being verified.

Steps 3 and 4 can be performed simultaneously if multiple incubators are available.

3. Invert the tubes to eliminate air trapped inside the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation. Check tubes for growth and gas production at 24 hours. If no change has occurred, continue incubation for another 24 hours.

If growth and gas are not produced in 48 ± 3 hours, the colony was not coliform. If growth and gas are produced in 48 ± 3 hours, use a sterile loop to inoculate one EC Medium Broth tube from each gas-positive LT Broth tube.

4. Invert the tubes to eliminate air trapped inside the inner vials. Incubate the EC Medium tubes at 44.5 ± 0.2 °C for 24 ±2 hours. After one hour, invert the tubes to remove trapped air in the inner vial.

5. Growth and gas production at 44.5 °C within 24 ± 2 hours confirms the presence of fecal coliforms.

Record the results of the test. See *Interpreting and reporting results*.

Interpreting and reporting results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as “Confluent Growth With or Without Coliforms.”
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Consumables and replacement items

Confirmation of fecal coliforms (m-FC or m-FC/RA)

Required media and reagents

Description	Unit	Catalog number
m-FC prepared agar plates	15/pkg	2811515
m-FC Broth Ampules, plastic	50/pkg	2373250
m-FC w/Rosolic Acid Broth Ampules, plastic	50/pkg	2428550
m-FC Broth PourRite™ Ampules (for fecal coliform presumptive)	20/pkg	2373220
m-FC with Rosolic Acid Broth PourRite™ Ampules (fecal coliform presumptive)	20/pkg	2428520

Required apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Counter, hand tally	1	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	1	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	1	2486100
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	1	54653
Forceps, stainless steel	1	2141100
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Inoculating Needle, disposable	25/pkg	2748925
Loop, inoculating, disposable	25/pkg	2749125
Microscope, compound	each	2942500

Optional media and reagents

Description	Unit	Catalog number
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Incubator, Water Bath, 110 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 220 VAC, 50/60 Hz	each	2616302

Confirmation of total coliforms (brilliant green bile broth and lauryl tryptose broth)

Required media and reagents

Description	Unit	Catalog number
Brilliant Green Bile Broth Tubes (for total coliform confirmation)	15/pkg	32215
Lauryl Tryptose Broth Ampules, sterile (for enrichment technique)	20/pkg	1472520
Lauryl Tryptose Broth Tubes, single-strength (for total coliform confirmation)	15/pkg	2162315

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	1	2087742
Ampule Breaker, PourRite™	each	2484600
Burner, Bunsen	each	2162700
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Isopropyl alcohol	500 mL	1445949
Loop, inoculating, disposable	25/pkg	2749125
Pad, absorbent, with dispenser	1000/pkg	1491800

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ID	3.7 m (12 ft)	56019
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	10/pkg	2097810

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