

m-FC and m-FC/RA Broth PourRite Ampules¹

Method 8074

Membrane Filtration

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

¹ USEPA approved 9222 D.



Test preparation

Before starting

Let the media in PourRite ampules increase to room temperature before the ampule is opened.

Set the temperature of the incubator to 44.5 ± 0.2 °C (112.1 ± 0.4 °F). Let the incubator temperature become stable, then add the samples.

To remove environmental *Klebsiella* from the fecal coliform population, increase the incubator temperature to 45.0 ± 0.2 °C (113 ± 0.4 °F).

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

Wash hands thoroughly with soap and water.

Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.

Make sure that all of the materials that come in contact with samples are sterile.

During filtration, remove the vacuum as soon as the funnel is empty so that the membrane filter does not become dry.

As an alternative to the filter assembly with flask, use a sterile, disposable filter unit.

As an alternative to the incubator, use a water bath with a rack. Put the petri dish in a sealed bag.

Items to collect

Description	Quantity
Broth ampule, m-FC	1
Sterile buffered dilution water	1
Membrane filter, 0.45 micron	1
Petri dish with absorbent pad, 47-mm	1
Filtration apparatus with aspirator or pump	1
Forceps, sterilized	1
Incubator	1
Microscope, low-power	1
Pipet(s) for dilution or for sample volumes less than 100 mL, if necessary	1

Refer to [Consumables and replacement items](#) on page 8 for order information.

Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. Keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

Sample volumes

Use a sample volume that is applicable to the sample type. For samples with a low level of bacteria such as finished, potable water, use 100 mL of sample. Use less sample for non-potable water or water that contains more bacteria.

When the approximate bacteria level is unknown, analyze three different sample volumes. Use the results from the sample volume that shows approximately 20 to 200 colonies for each membrane filter.

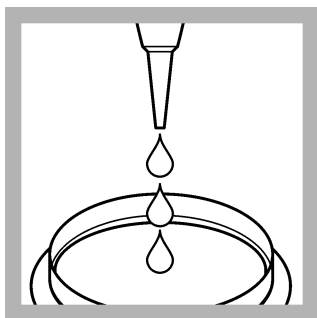
When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

Sample dilution

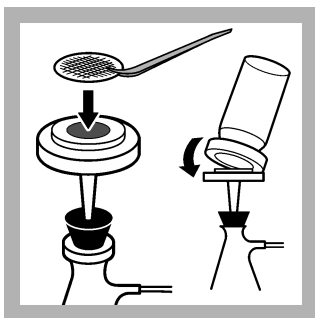
Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

1. Wash hands thoroughly with soap and water.
2. Invert the sample container for 30 seconds (approximately 25 times).
3. Open a bottle of sterile buffered dilution water.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert for 30 seconds (25 times). This is a 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. If necessary, continue to dilute the sample.

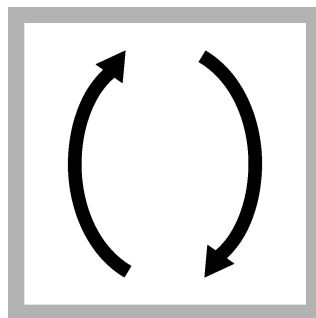
Presumptive test for fecal coliforms



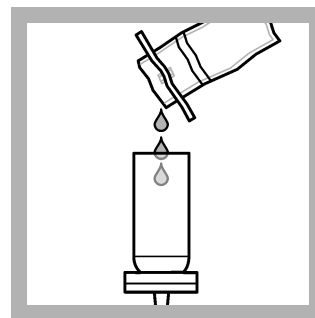
1. Invert one m-FC PourRite broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



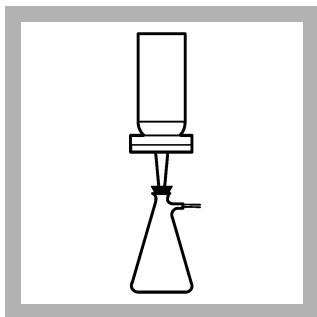
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



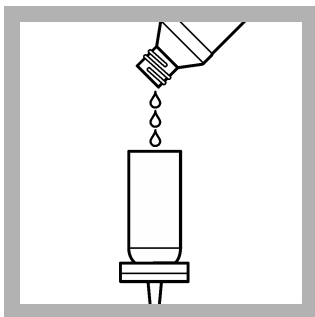
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



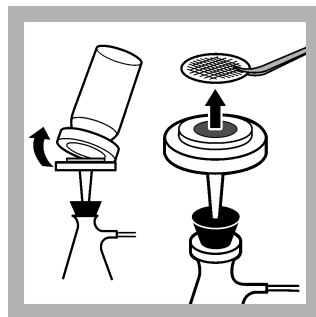
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



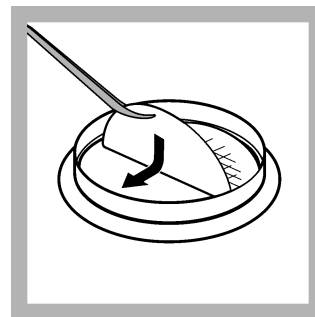
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



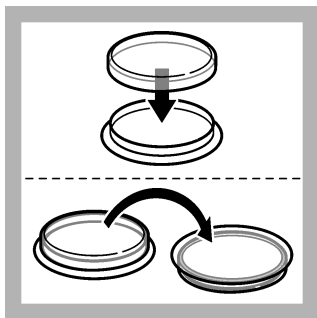
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



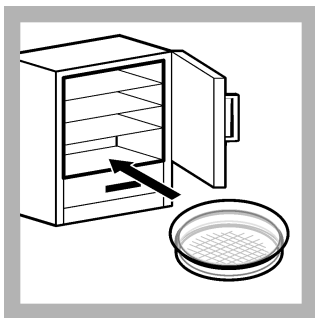
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



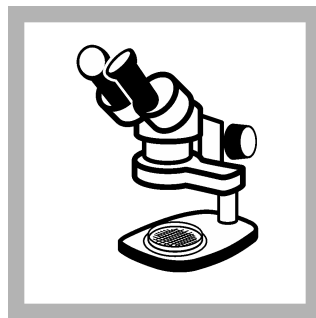
8. Put the membrane filter on the absorbent pad. Let the membrane filter bend and fall equally across the absorbent pad to make sure that air bubbles are not caught below the filter.



9. Put the lid on the petri dish and invert the petri dish.



10. Incubate the inverted petri dish at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 (± 2) hours.



11. Remove the petri dish from the incubator. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter. Refer to [Interpret and report the coliform results](#) on page 7.

About confirmation of total coliforms

For potable water samples, do the confirmation procedure on typical colonies to make sure that they are coliforms. Confirm sheen colonies to a maximum of five. Move growth from each colony to inoculate parallel tubes of Lauryl Tryptose (LT) single-strength (SS) broth and Brilliant Green Bile (BGB) broth. Growth and gas production in the two tubes makes sure that the organisms are coliforms. Most Probable Number (MPN) coliform tubes are recommended for this procedure.

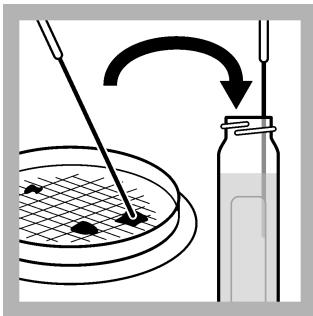
Use the swabbing technique for fecal coliforms or *E. coli* as follows:

- To determine only if total coliforms are in or not in the sample
- To inoculate EC or EC/MUG media

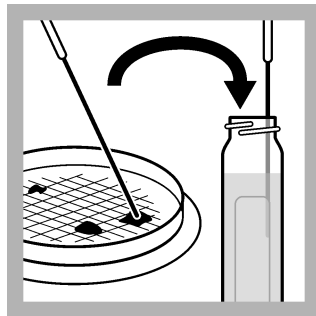
Inoculate in the sequence that follows:

1. EC or EC/MUG media
2. Lauryl Tryptose (LT) single-strength broth
3. Brilliant Green Bile (BGB) broth

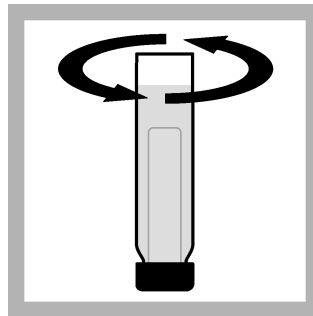
Confirmation test of total coliforms (LT and BGB)



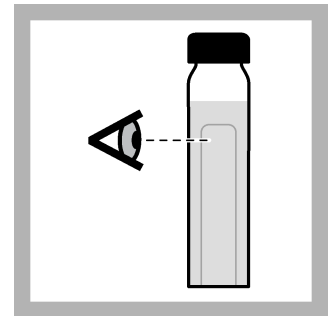
1. Touch a sterilized inoculating needle or a sterile disposable needle to the coliform (sheen) colony growth. Put the needle in a Lauryl Tryptose broth tube.



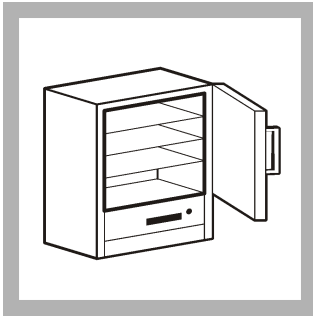
2. Touch the sterilized inoculating needle again to the same coliform (sheen) colony growth. Put the needle in a Brilliant Green Bile (BGB) broth tube.



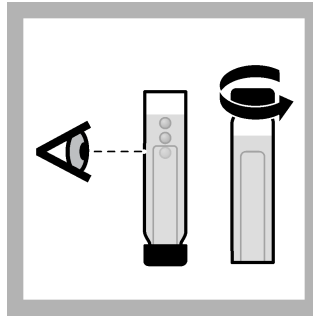
3. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



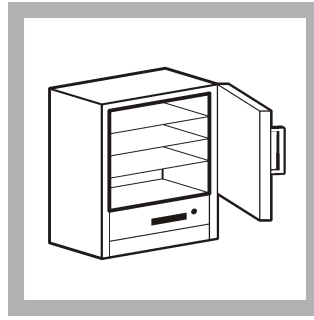
4. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



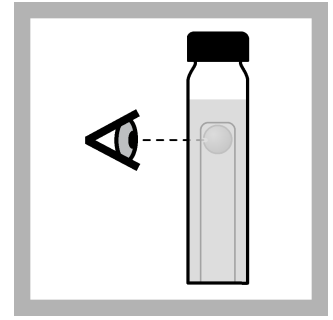
5. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



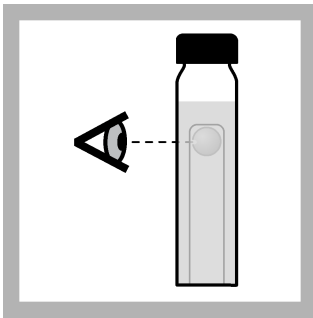
6. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



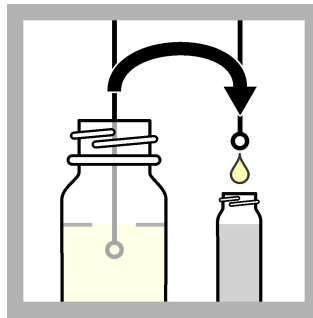
7. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 (± 2) hours. **Note:** *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



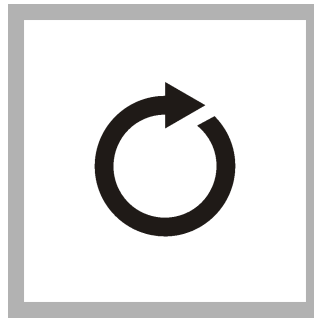
8. After 24 (± 2) hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria. If no gas can be seen, put the tubes in the incubator for 24 (± 2) hours (48 (± 3) hours total) and examine the tubes again.



9. After 48 (± 3) hours, gently tap each tube and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria. If none of the tubes contain gas, then the test is negative for total coliform bacteria.

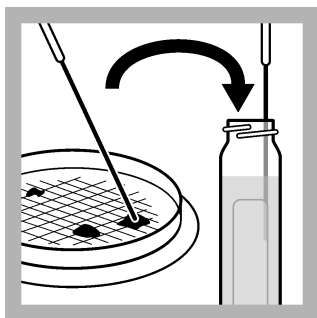


10. Confirm positive results. If growth and gas occur in the Lauryl Tryptose broth tube but not in the Brilliant Green Bile (BGB) broth tube, inoculate another Brilliant Green Bile (BGB) broth tube from the gas-positive Lauryl Tryptose broth tube.

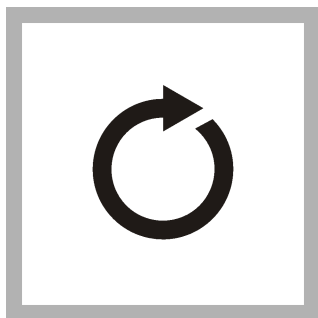


11. Do steps 3–9 again on the Brilliant Green Bile (BGB) broth tube. If growth and gas occur within 48 (± 3) hours, the colony is confirmed as coliform.

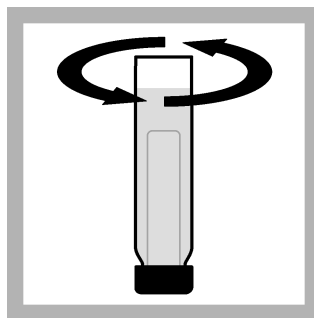
Confirmation of fecal coliforms (LT and EC)



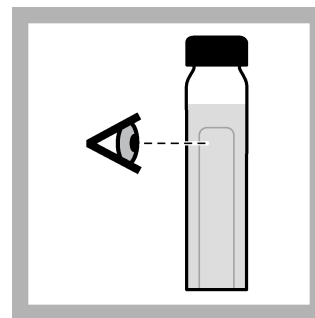
1. Touch a sterilized inoculating needle or a sterile disposable needle to a typical blue colony. Put the needle in a Lauryl Tryptose broth tube.



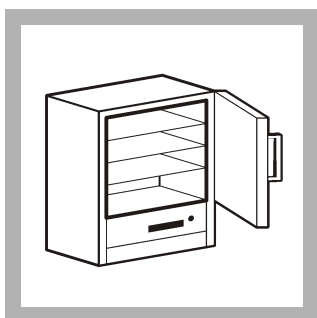
2. Do step 1 again for each test being verified. Use one Lauryl Tryptose broth tube for each test.



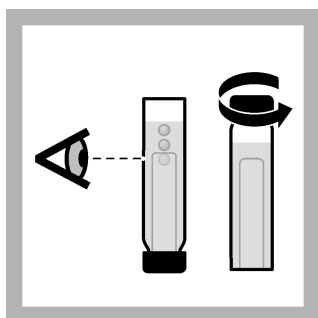
3. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



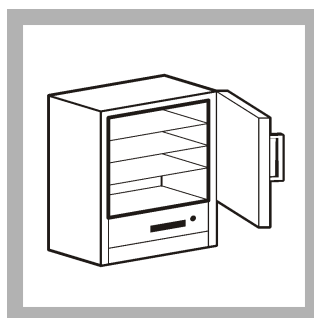
4. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



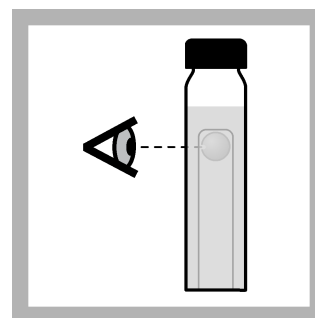
5. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



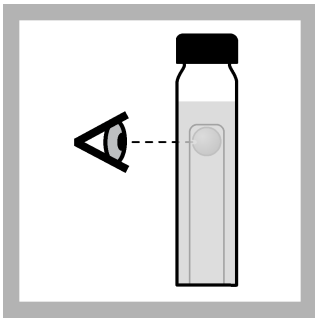
6. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



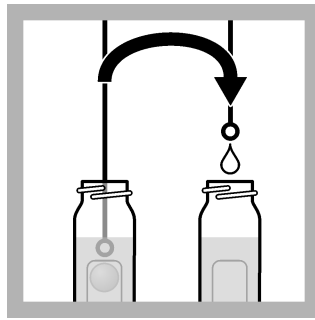
7. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 (± 2) hours. **Note:** *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



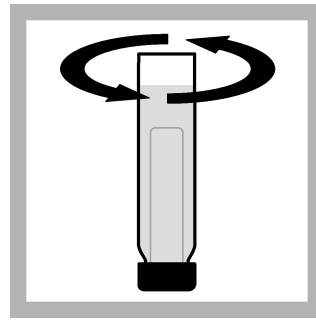
8. After 24 (± 2) hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria. If no gas can be seen, put the tubes in the incubator for 24 (± 2) hours (48 (± 3) hours total) and examine the tubes again.



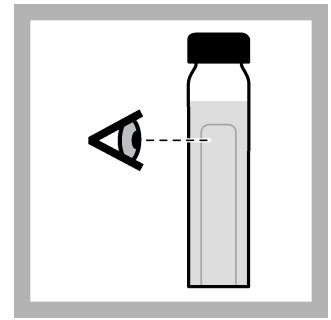
9. After 48 hours (± 3) hours, gently tap each tube and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for fecal coliform bacteria. If none of the tubes contain gas, then the test is negative for fecal coliform bacteria.



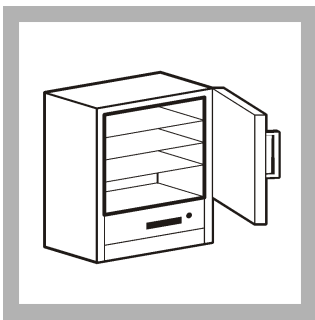
10. If the inner vial contains gas bubbles, use a sterile loop to inoculate an EC Medium Broth tube from the gas-positive Lauryl Tryptose broth tube.



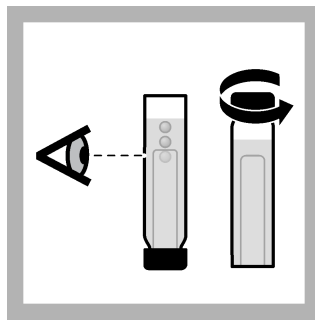
11. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



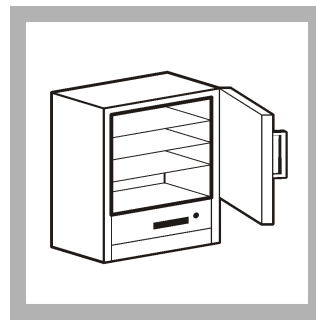
12. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



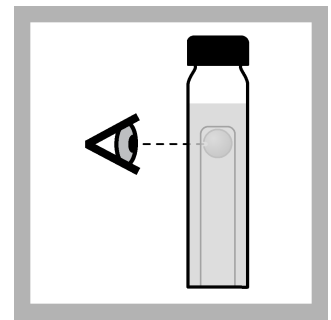
13. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.5 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



14. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



15. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 32.36 °F) for 24 (± 2) hours.



16. After 24 (± 2) hours, remove the tubes from the incubator. Tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for fecal coliform bacteria. If no gas is seen, the test is negative for fecal coliform bacteria.

Interpret and report the coliform results

Report the coliform density as the number of colonies in 100 mL of sample. For total coliforms, use a sample volume that gives 20–80 coliform colonies on the membrane filter. For fecal coliforms, use a sample volume that gives 20–60 fecal coliform colonies on the membrane filter.

If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the coliform density determination.

1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated coliform colonies.
2. Determine the coliform density as follows:

Membrane filter(s)	Coliform density determination
One membrane filter	Coliform colonies in 100 mL = Coliform colonies counted ÷ mL sample × 100 <i>Example: 50 coliform colonies were counted. The sample volume was 20 mL. The coliform density is 50 ÷ 20 mL × 100 = 250 coliforms in 100 mL of sample.</i>
Multiple filters, dilutions or duplicates for each sample	Average coliform colonies in 100 mL = Sum of coliform colonies in all samples ÷ sum of mL sample × 100 <i>Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The coliform density is (5 + 9) ÷ (50 + 50) × 100 = 14 coliforms in 100 mL of sample.</i>

3. If colonies are not isolated or if there are more than 200 colonies of all types:
 - a. Report the results as “Confluent growth with or without coliforms” when the bacteria grows together across some or all of the membrane filter.
 - b. Do the test procedure again with half the sample volume. If the total number of colonies (coliforms plus non-coliforms) is more than 200 for each membrane or the colonies are not isolated, report the results as “Too numerous to count” (TNTC).
 - c. Do the test procedure again with a dilution that gives approximately 50 coliform colonies and not more than 200 colonies of all types.

Controls for coliform bacteria tests

Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive control.

Summary of method

A fecal coliform test is usually done on wastewater, river, bathing, and other non-potable waters. A special medium and an elevated incubation temperature prevent growth of non-fecal coliforms. Fecal coliforms that grow on the membrane form an acid that reacts with an aniline dye in the medium. A blue color forms.

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

The membrane filtration procedure is used for samples that are low in turbidity and have low bacteria counts. The sample is poured through a membrane filter. The bacteria in the sample stays on the membrane filter. The membrane filter is moved to a petri dish that contains a nutritional broth or agar. During incubation, the bacteria grow and form colonies on the membrane filter. After incubation, the filter is examined with a microscope for bacteria colonies.

Consumables and replacement items

Presumptive for fecal coliforms (m-FC or m-FC/RA)

Required reagents

Description	Quantity/test	Unit	Item no.
m-FC prepared agar plates	1	15/pkg	2811515
m-FC broth ampules, plastic	1	50/pkg	2373250
m-FC with Rosolic Acid broth ampules, plastic	1	50/pkg	2428550

Presumptive for fecal coliforms (m-FC or m-FC/RA) (continued)

Description	Quantity/test	Unit	Item no.
m-FC broth PourRite™ ampules (for fecal coliform presumptive)	1	20/pkg	2373220
m-FC with Rosolic Acid broth PourRite™ ampules (for fecal coliform presumptive)	1	20/pkg	2428520
Dilution water, buffered, 99 mL, sterile ¹	1	25/pkg	1430598

Required apparatus

Description	Unit	Item no.
Ampule breaker, PourRite™	each	2484600
Membrane filter holder, magnetic, 300-mL funnel	each	1352900
Filter pump, aspirator	each	213100
Flask, filtering, glass, 1000 mL	each	54653
Forceps, stainless steel	each	2141100
Membrane filter, 0.45 micron, 47 mm diameter, sterile	200/pkg	1353001
Membrane filter, 0.45 micron, 47 mm diameter, sterile EO (ethylene oxide)	150/pkg	2936100
Microscope, compound	each	2947050
Petri dish with absorbent pad, for 47-mm membrane filters, sterile	100/pkg	1471799
Petri dish with absorbent pad, for 47-mm membrane filters, sterile EO (ethylene oxide)	150/pkg	25248000
Stopper, rubber, size 8, for filtration assembly	6/pkg	211908
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Tubing, rubber, 7.9 mm (5/16-in.) inside diameter	3.66 m (12 ft)	56019

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Portable incubator with 12 VDC power socket	each	2569900
AC power supply for portable incubator, 110–240 VAC	each	2968100
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Portable incubator rack, general purpose/petri dish	each	2580502

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012

¹ Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Sample collection (continued)

Description	Unit	Item no.
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

Optional reagents and apparatus

Description	Unit	Item no.
Disposable filter funnels with membrane filters, sterile	150/pkg	2586300
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet, serological, 2 mL, sterile, glass	35/pkg	2093136
Pipet filler, safety bulb	each	1465100
Support base for disposable filter funnels	each	2586201
Vacuum pump, hand-operated	each	1428300

Confirmation of total coliforms (BGB and LT)

Note: Many of the confirmation products are given in the presumptive products tables.

Required reagents

Description	Quantity/test	Unit	Item no.
Brilliant Green Bile (BGB) Broth tubes (for total coliform confirmation)	1	15/pkg	32215
Lauryl Tryptose Broth tubes, single-strength (for total coliform confirmation)	1	15/pkg	2162315

Required apparatus

Note: Many of the required apparatus are in the required apparatus table for confirmation of fecal coliforms (EC medium broth).

Description	Quantity/test	Unit	Item no.
Inoculating loop, plastic disposable	1	25/pkg	2749125
Inoculating loop, nichrome wire	1	each	2112100



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