Coliforms, Total, Fecal and *E. coli*

DOC316.53.01224

m-Endo Broth Ampule[1]

Method 8074

Membrane Filtration

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

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 9222 B and 9221 B.

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 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Potable water must have no coliform bacteria. Do not dilute potable water samples.
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 m-Endo broth, use m-Endo agar plates.

Items to collect

Description	Quantity
Broth ampule, m-Endo	1
Confirmation media	varies
Sterile buffered dilution water	varies
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 10 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.

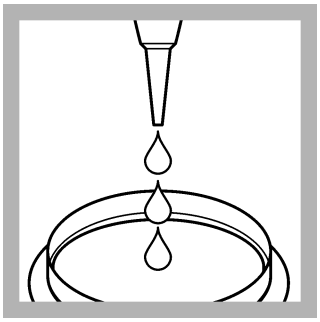
When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

Sample dilution

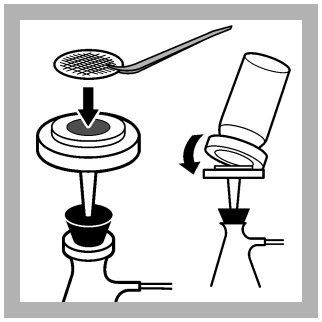
Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

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.

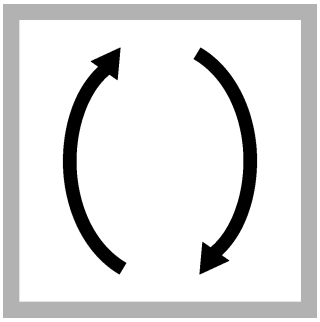
Presumptive test for total coliforms



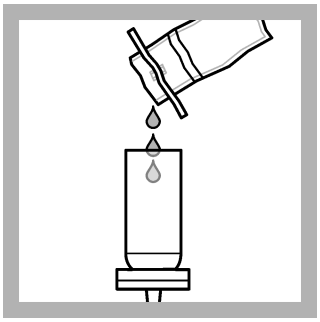
1. Invert one m-Endo broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



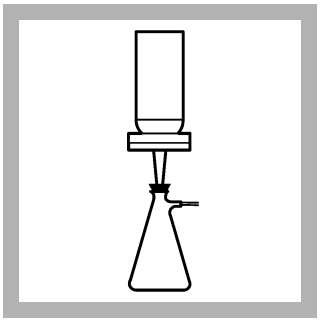
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



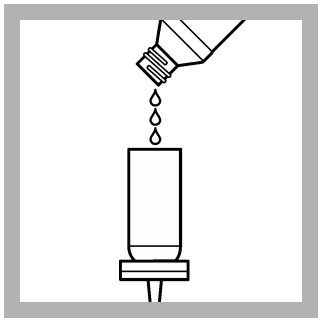
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



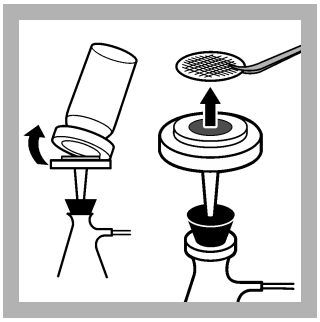
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



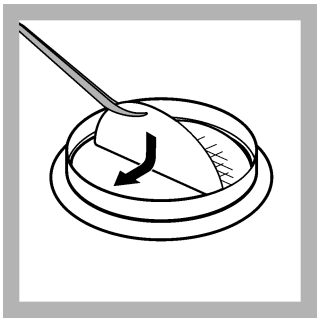
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



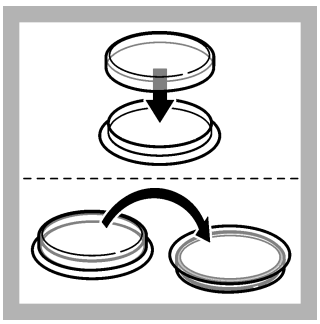
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



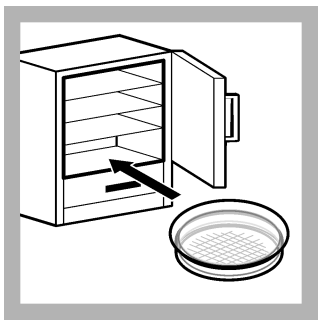
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



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 35 ± 0.5 °C (95 ± 0.9 °F) for 22–24 hours.

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.

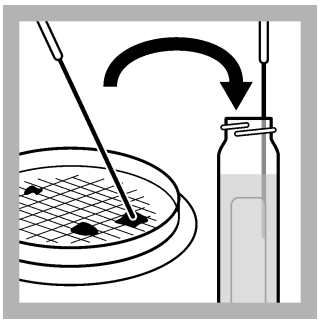
Use the swabbing technique for fecal coliforms or *E. coli* as follows:

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

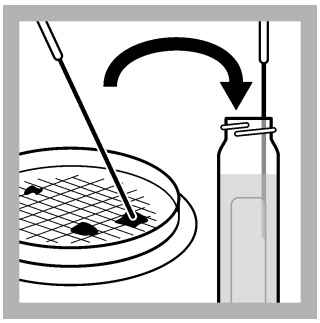
Inoculate in the sequence that follows:

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

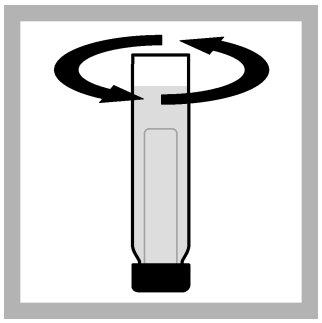
Confirmation test of total coliforms (LT and BGB)



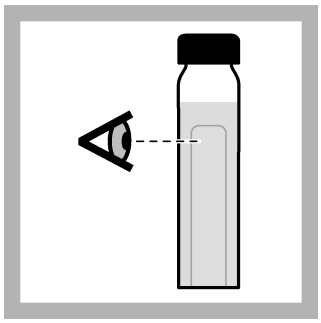
1. Touch a sterilized inoculating needle or a sterile disposable needle to the coliform (sheen) colony growth. Put the needle in a Lauryl Tryptose broth tube.



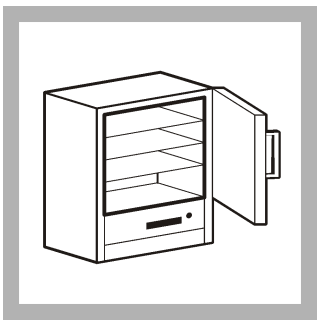
2. Touch the sterilized inoculating needle again to the same coliform (sheen) colony growth. Put the needle in a Brilliant Green Bile (BGB) broth tube.



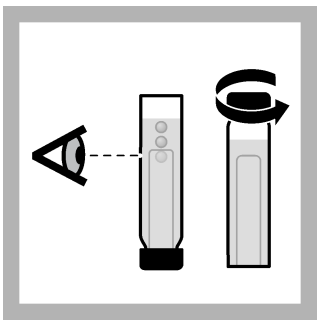
3. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



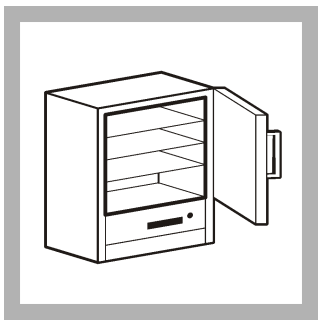
4. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



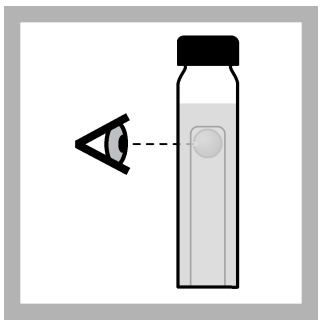
5. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



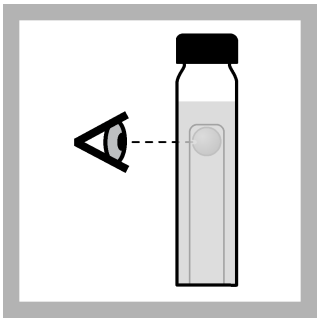
6. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



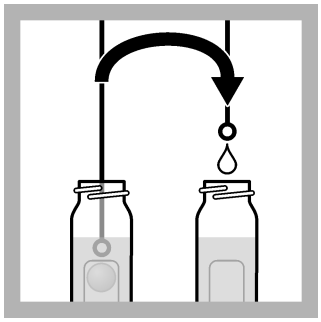
7. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



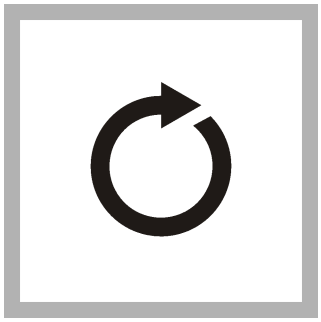
8. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be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

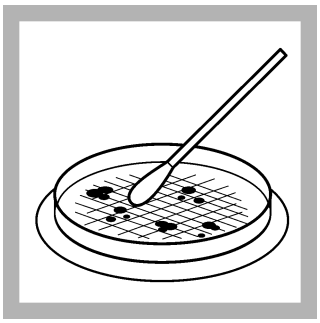


10. Confirm positive results. If growth and gas occur in the Lauryl Tryptose broth tube but not in the Brilliant Green Bile (BGB) broth tube, inoculate another Brilliant Green Bile (BGB) broth tube from the gas-positive Lauryl Tryptose broth tube.

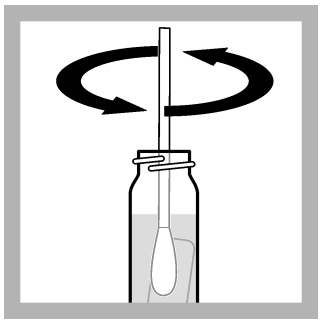


11. Do steps 3–9 again on the Brilliant Green Bile (BGB) broth tube. If growth and gas occur within 48 ± 3 hours, the colony is confirmed as coliform.

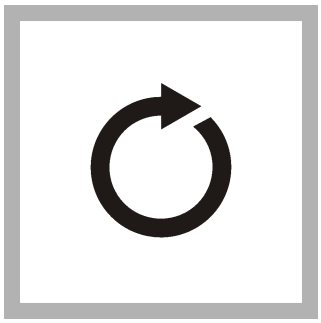
Confirmation test for fecal coliforms (EC Medium)



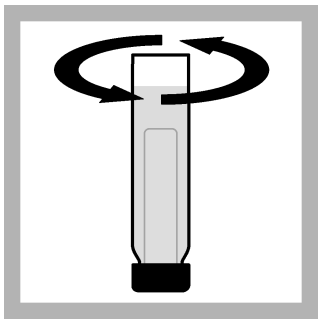
1. Use a sterile cotton swab or inoculating loop to touch all of the surface of the membrane filter that is positive for total coliforms.



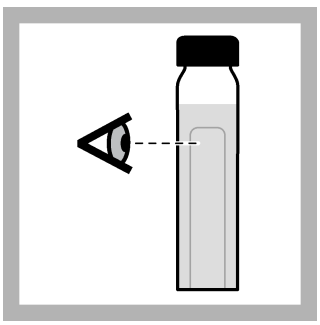
2. Swirl the cotton swab or inoculating loop in an EC Medium Broth tube to move the colonies collected from the filter to the tube.



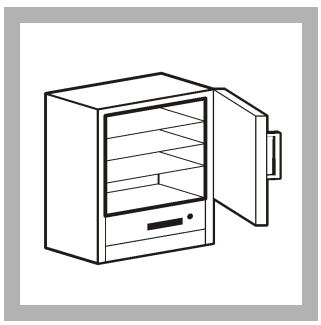
3. Do steps 1–2 again for each test to be verified. Use one broth tube for each test. Use the same cottom swab.



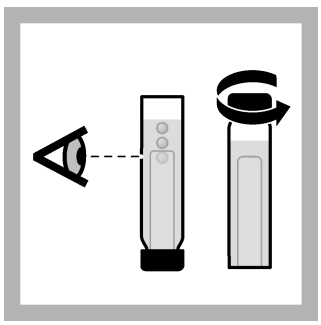
4. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



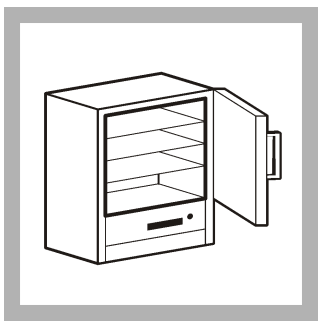
5. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



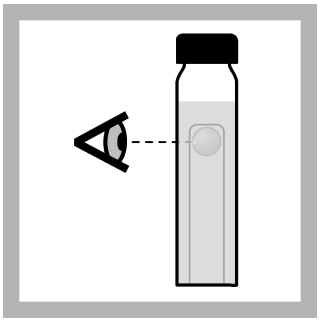
6. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



7. 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.



8. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 ± 2 hours.



9. After 24 ± 2 hours, remove the samples from the incubator. 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.

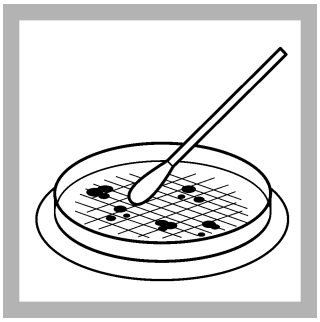
Confirmation of *E. coli* (EC or EC/MUG)

⚠CAUTION

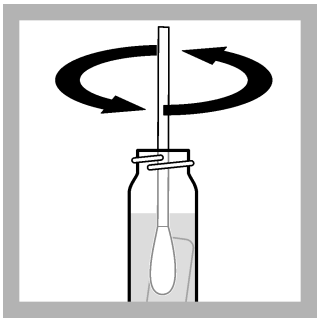
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

When the nutritional media contains MUG, use a long-wave (e.g., 365 nm) UV lamp to confirm the presence of *E. coli*. The sample will fluoresce if *E. coli* is in the sample. No additional confirmation procedure is necessary.

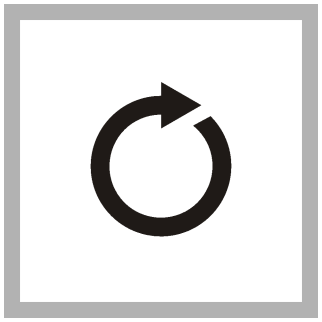
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



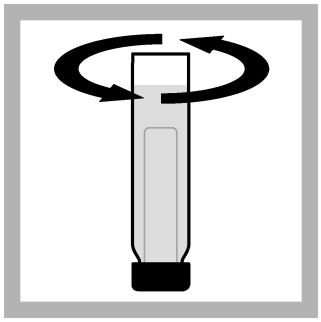
1. Use a sterile cotton swab or inoculating loop to touch all of the surface of the membrane filter that is positive for total coliforms.



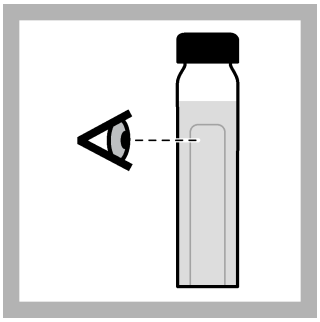
2. Swirl the cotton swab or inoculating loop in an EC/MUG Broth tube to move the colonies collected from the filter to the tube.



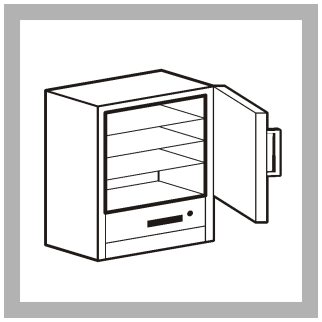
3. Do steps 1–2 again for each test to be verified. Use one broth tube for each test. Use the same cottom swab.



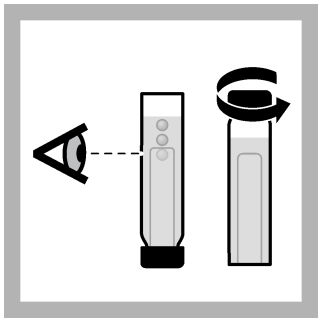
4. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



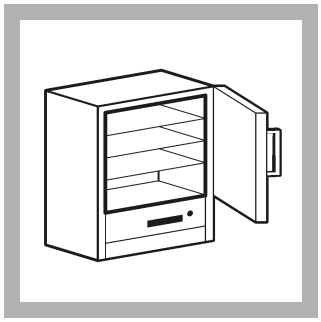
5. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



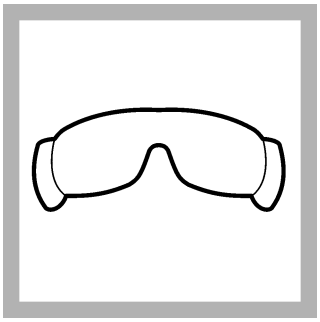
6. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



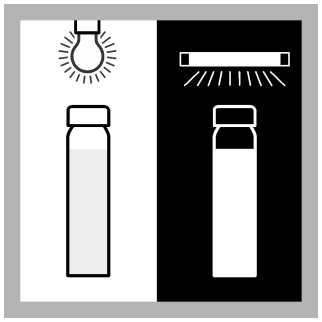
7. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



8. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 ± 2 hours.



9. Put on UV safety goggles



10. Apply UV light to the incubated sample that contains MUG broth with a long-wave UV lamp. Examine the tubes in a dark area. Look at the tube 90° from the UV light. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* positive confirmation.
If the sample fluoresces, *E. coli* bacteria are in the sample.
If the sample does not fluoresce, the test is negative for *E. coli*.

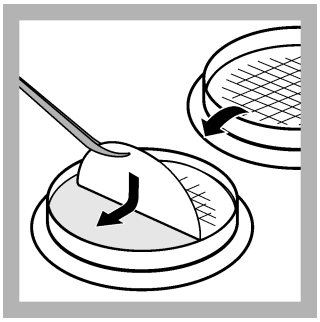
Confirmation of E. coli (Nutrient Agar/MUG)

⚠CAUTION

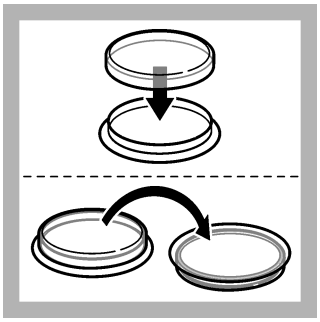
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

When the nutritional media contains MUG, use a long-wave (e.g., 365 nm) UV lamp to confirm the presence of *E. coli*. The sample will fluoresce if *E. coli* is in the sample. No additional confirmation procedure is necessary.

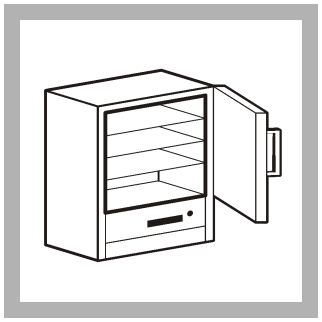
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



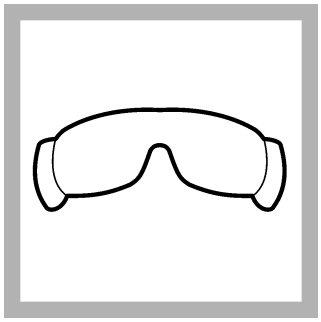
1. Use sterile forceps to put the membrane filter that is positive for total coliforms on the prepared NA/MUG agar plate. Let the membrane filter bend and fall equally across the agar to make sure that air bubbles are not caught below the filter.



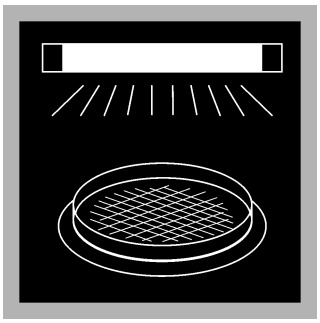
2. Put the lid on the petri dish and invert the petri dish.



3. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 4 hours.



4. Put on UV safety goggles



5. Remove the lid from the petri dish. Apply UV light to the colonies in a dark area. If the colonies fluoresce, *E. coli* bacteria are in the sample.
If the colonies do not fluoresce, the test is negative for *E. coli*.

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 *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.*
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 *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.*

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

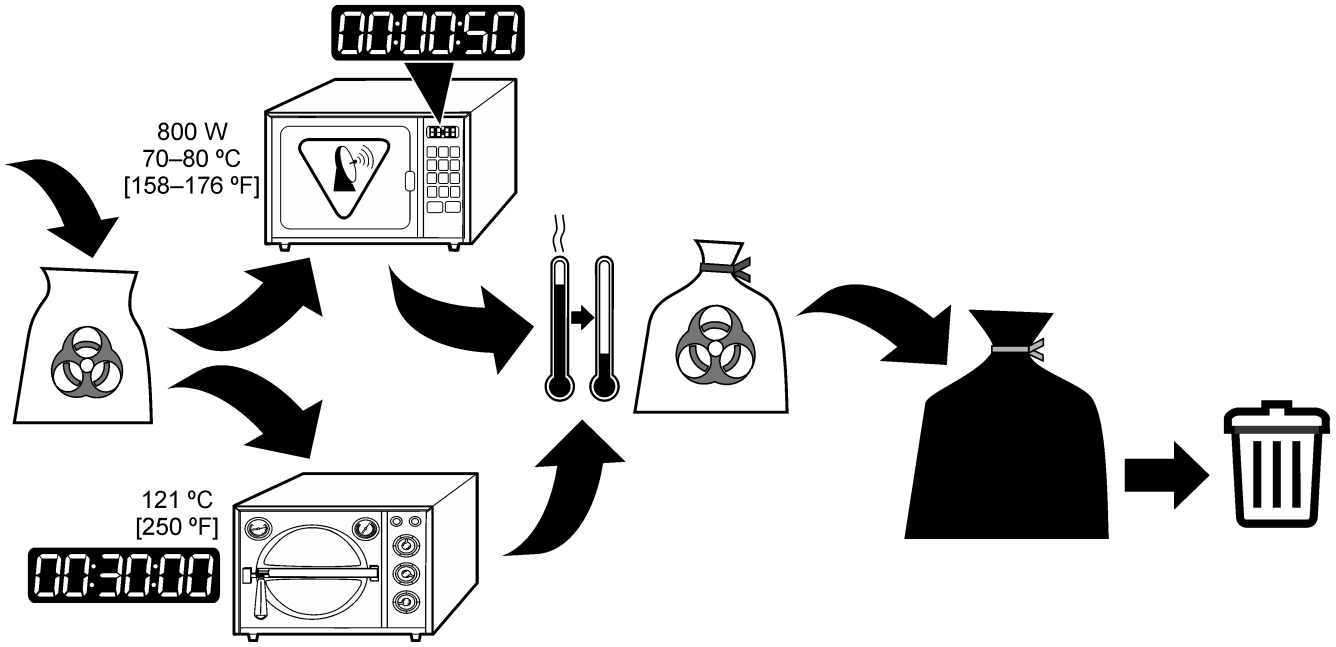
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive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

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1 and the information that follows.

- Microwave—Add 1–2 mL of hypochlorite (bleach) solution to each test container. If a container has a lid, do not close it too tightly. Put the container in the microwave at 70–80 °C (158–176 °F) for 50 seconds. Wait 10 to 15 minutes. Pour the liquid down the drain.
- Autoclave—Put the used test containers in a contaminated items bag or biohazard bag to prevent leaks. Do not seal the bag. Put the bag in the autoclave at 121 °C (250 °F) for 30 minutes at 1.0 bar (15 psi) of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

Figure 1 Bacteria disposal



Summary of method

Coliforms ferment lactose in the medium and form an acid-aldehyde complex. The complex mixes with Schiff's Reagent (also in the medium) to form an iridescent green coating above the colonies. When magnified 10x to 15x, coliforms show as dark red colonies with a greenish-gold sheen.

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 total coliforms (BGB and LT)

Required reagents

Description	Quantity/test	Unit	Item no.
m-Endo broth ampules, plastic	1	50/pkg	2373550
m-Endo broth PourRite™ ampules, glass (for total coliform presumptive)	1	20/pkg	2373520
m-Endo, prepared agar plates	1	15/pkg	2811615
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

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

Required apparatus (continued)

Description	Unit	Item no.
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
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.
m-Endo broth, glass bottle	100 mL	2373542
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

Optional reagents and apparatus (continued)

Description	Unit	Item no.
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

Confirmation of fecal coliforms (EC medium broth)

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

Required reagents

Description	Quantity/test	Unit	Item no.
EC Medium Broth tubes (for fecal coliform confirmation)	1	15/pkg	1410415

Confirmation of *E. coli* with EC/MUG

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

Required reagents

Description	Quantity/test	Unit	Item no.
EC Medium with MUG Broth Tubes (for E. coli confirmation)	1	15/pkg	2471515

Required apparatus

Description	Quantity/Test	Unit	Item no.
UV lamp, long-wave, portable, 4 watt	1	each	2415200
Replacement bulb for portable UV lamp	1	each	2584600
UV lamp, long-wave, 115 VAC	1	each	2184300
UV lamp, long-wave, 230 VAC	1	each	2184302
UV blocking eyewear	1	each	SM730-1033

Optional apparatus

Description	Unit	Item no.
E. coli fluorescence standard	each	2361100
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302

Confirmation of *E. coli* with nutrient agar

Note: *Many of the confirmation products are given in the presumptive products tables and in the products tables for the confirmation of E. coli with EC/MUG.*

Required reagents

Description	Quantity/test	Unit	Item no.
Nutrient agar with MUG prepared plates	1	15/pkg	2812115
Nutrient agar with MUG tubes, two tests for each tube (for *E. coli* confirmation)	1	6/pkg	2437306

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