Coliforms—Total, Fecal and *E. coli*

DOC316.53.001224

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

m-Endo

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.

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 absorbent pad (in a petri dish) saturated with a culture medium that is selective for coliform growth. The petri dish containing the filter and pad 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:

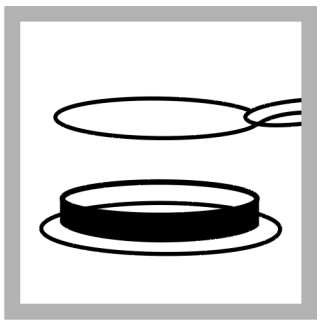
When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a 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.
Potable water must not contain any coliform bacteria. Do not dilute potable water samples.
Prepared m-Endo agar plates may be used instead of m-Endo broth.

Potable water procedures

To test potable water with the MF Method, examine a 100-mL sample for total coliforms by incubating a filter at 35 ± 0.5 °C for 22–24 hours on m-Endo Broth. Coliforms ferment lactose in the medium and produce an acid-aldehyde complex. This complex combines with Schiff's Reagent (also in the medium) to form an iridescent green coating over the colonies. When magnified 10 to 15 times, coliforms appear as dark red colonies with a greenish-gold sheen.

For replacement items, see *m-Endo Broth for presumptive total coliforms*.

Presumptive test for total coliforms (m-Endo), method 8074



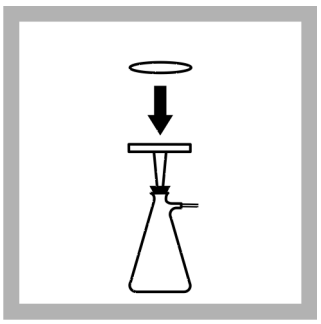
1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the lid.

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.

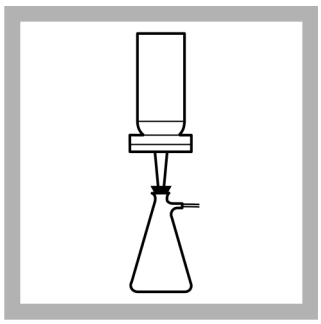
For ease of use, petri dishes with pads are available.

2. Invert an m-Endo Broth PourRite Ampule 2 to 3 times to mix the broth. Use the ampule breaker to break open the ampule. Carefully pour the contents evenly over the absorbent pad. Replace the petri dish lid. Repeat steps 1 and 2 for each petri dish being prepared.



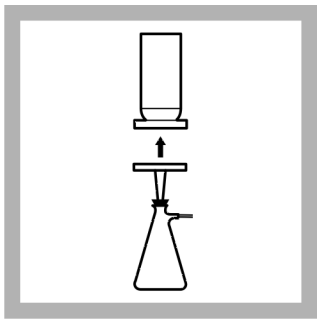
3. Set up the Membrane Filter Assembly. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.

Alternatively, a sterile disposable filter unit may be used.

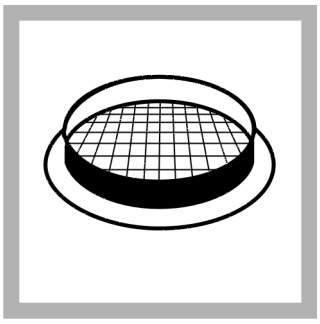


4. Invert the sample for 30 seconds to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample. Release the vacuum. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Repeat rinsing step 2 more times.

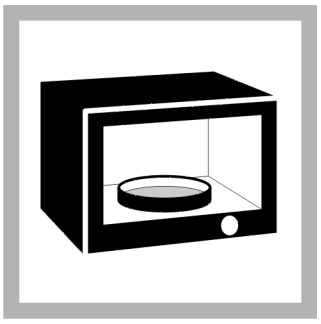
Release the vacuum when the filter is dry to prevent damage to the filter.



5. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the filter immediately 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 filter touches the entire pad. Replace the petri dish lid.

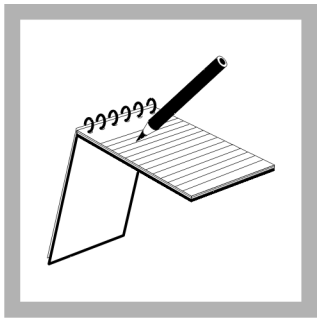


7. Invert the petri dish and incubate at 35 ± 0.5 °C for 22–24 hours.

8. After incubating, use a 10 to 15X microscope to count the red colonies that have a greenish-gold metallic sheen.

The sheen may extend over the entire colony, or it may be localized to the edge or to the center.

Presumptive test for total coliforms (m-Endo), method 8074 (continued)



9. Record the results of the test. See *Interpreting and Reporting Results*

Depending on the test protocol, confirm positive results.

To confirm total coliforms, follow *Confirmation of total coliforms (LT and BGB), method 8074*.

To confirm fecal coliforms, follow *Confirmation of fecal coliforms (EC medium), method 8074*.

To confirm *E. coli*, follow *Confirmation of E. coli (EC or EC/MUG), 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*:

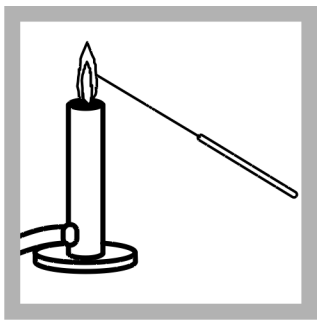
- When determining only the presence or absence of total coliforms
- When inoculating EC or EC/MUG media

Inoculate in this order:

1. EC or EC/MUG
2. LT SS Broth
3. BGB

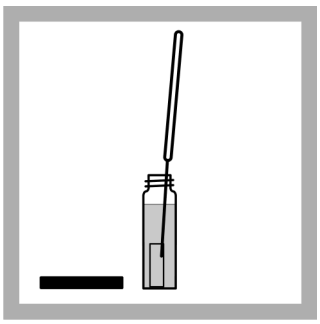
For replacement items, see *Confirmation of total coliforms (brilliant green bile broth and lauryl tryptose broth)*.

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

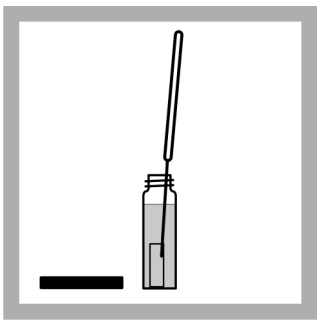


1. Sterilize an inoculating needle, or use a sterile, disposable inoculating needle.

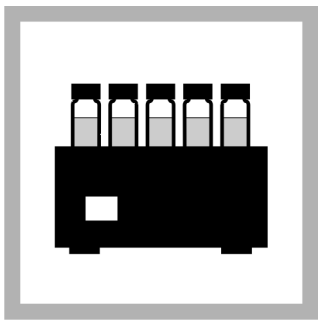
To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.



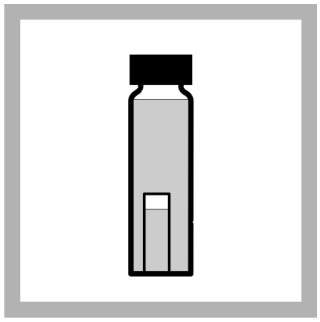
2. Touch the needle to the coliform (sheen) colony grown on m-Endo plate. Transfer to a single-strength Lauryl Tryptose (LT) Broth tube.



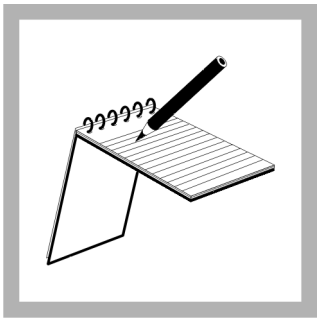
3. Again touch the same coliform colony with the needle. Transfer to a Brilliant Green Bile (BGB) Broth tube.



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*

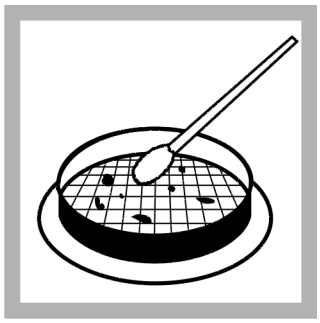
Confirm positive results. If growth and gas are produced in the LT Broth tube but not in the BGB Broth tube, inoculate another BGB tube from the gas-positive LT Broth tube. Incubate this BGB Broth tube and check for growth and gas after 24 hours and/or after 48 hours. If growth and gas are produced within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

Confirmation of fecal coliforms (EC medium)

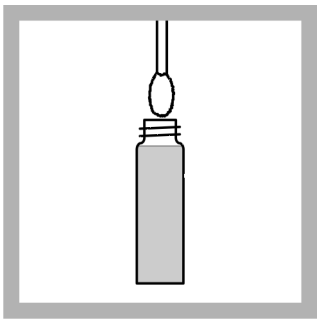
Analyze total-coliform-positive potable water samples for the presence of fecal coliform or *E. coli*. Confirm fecal coliforms from a membrane filter positive for total coliforms by swabbing the membrane with a sterile cotton swab and inoculating a tube of EC Medium Broth. Growth and gas production in the EC Medium confirms the presence of fecal coliforms.

For replacement items, see *Confirmation of fecal coliforms (EC medium broth)*.

Confirmation of fecal coliforms (EC medium), method 8074

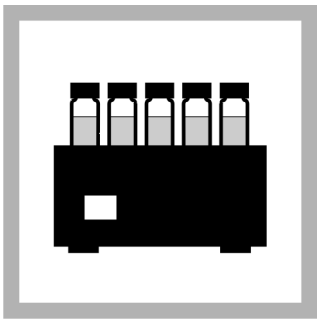


1. Use a sterile cotton swab or inoculating loop to swab the entire surface of the total coliform-positive membrane filter (colonies grown on m-Endo Broth).

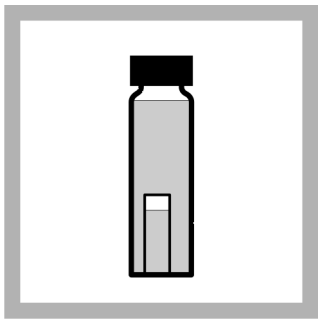


2. Swirl the cotton swab in an EC Medium Broth tube to transfer the colonies collected from the filter. Remove the cotton swab from the medium.

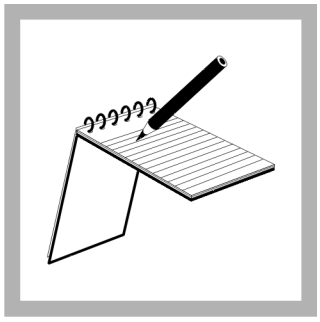
Use the same cotton swab to transfer colonies from the same petri dish to other broth media if desired.



3. Invert the tubes to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C. After one hour, Invert the tubes to remove trapped air in the inner vial and continue incubation.



4. After 24 ± 2 hours, check the inner vial for gas bubbles. Growth and gas bubbles in the EC Medium Broth tube confirm the presence of fecal coliforms.



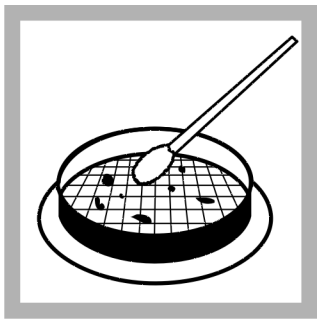
5. Record the results of the test. See *Interpreting and Reporting Results*.

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

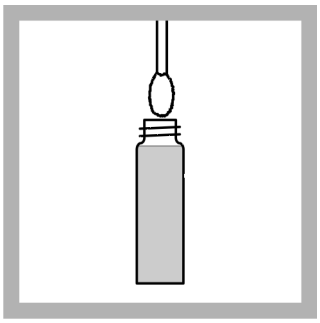
Potable water samples that test positive for total coliforms may be analyzed for the presence of *E. coli* in lieu of fecal coliforms. Use either EC medium or EC with MUG broth to confirm the presence of *E. coli* on a membrane filter positive for total coliforms.

For replacement items, see *E. coli confirmation with EC/MUG* and *E. coli confirmation with nutrient agar*.

Confirmation of *E. coli* (EC or EC/MUG), method 8074

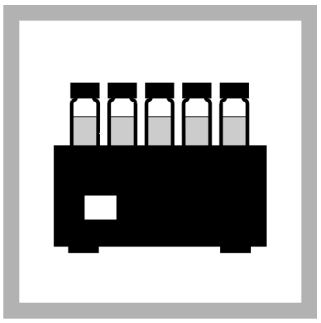


1. Use a sterile cotton swab or inoculating loop to swab the entire surface of the membrane filter that is positive for total coliforms (colonies grown on m-Endo Broth).

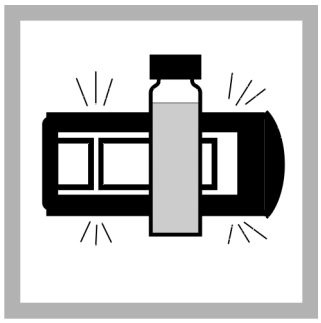


2. Swirl the cotton swab in an EC/MUG Broth tube to transfer the colonies collected from the filter. Remove the cotton swab from the medium.

Use the same cotton swab to transfer colonies from the same petri dish to other broth media if desired.



3. Invert the tubes to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation.



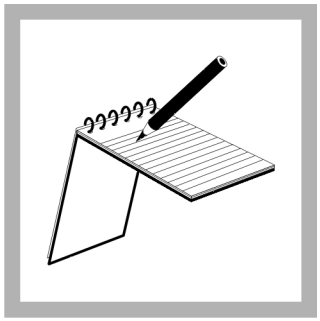
4. After 24 ± 2 hours, use a long-wave UV lamp to check the tube for fluorescence. Growth and fluorescence indicate the presence of *E. coli*.

Some glass will auto-fluoresce. Use Hach brand MPN tubes for best results.

Do not look directly through the MPN tube into the UV lamp. View the tube at 90° from the lamp. Examine the tubes in a darkened area.

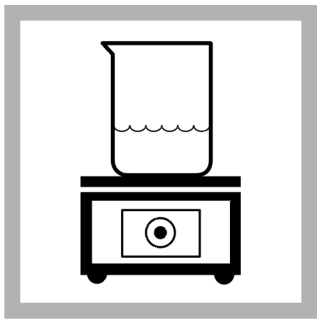
Have a fluorescent-positive and a fluorescent-negative tube, both with turbidity, to compare with the sample tube. Alternatively use an *E. coli* presence standard.

Confirmation of *E. coli* (EC or EC/MUG), method 8074 (continued)

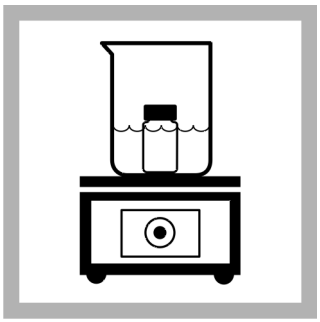


5. Record the results of the test. See *Interpreting and Reporting Results*.

Confirmation of *E. coli* (Nutrient Agar/MUG), method 8074



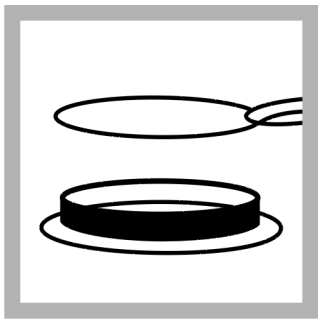
1. Heat a beaker of water, or a water bath, but do not allow it to boil.



2. Loosen the cap on one or more NA/MUG nutrient agar tubes. Place the tubes into hot water. When the agar melts, carefully remove the tubes from the water with tongs.

Pre-poured agar plates can also be used.

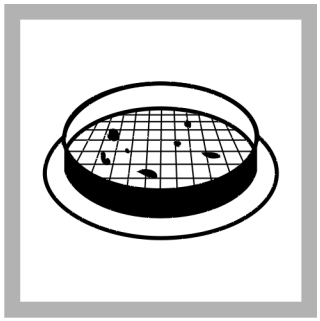
3. Using sterile technique, pour half of the contents of the tube into a sterile 47-mm petri dish. Immediately replace petri dish lid and allow agar to solidify undisturbed.



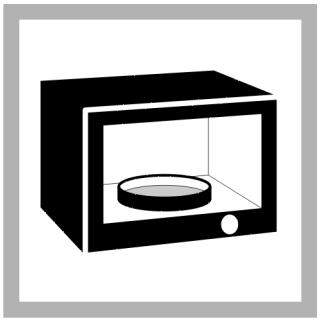
4. Use sterilized forceps to lift the membrane filter with total coliform colonies off the m-Endo absorbent pad.

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

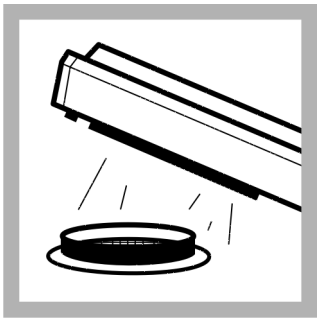
Confirmation of *E. coli* (Nutrient Agar/MUG), method 8074 (continued)



5. Immediately transfer the membrane filter to the petri dish containing NA/MUG. With a slight rolling motion, center the filter, grid side up, on the agar. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.



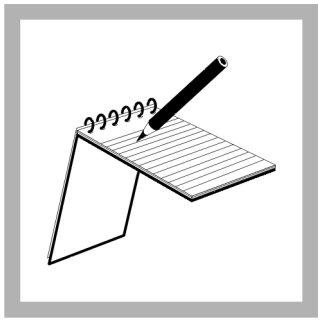
6. Invert the petri dish. Incubate for 4 hours at 35 ± 0.5 °C.



7. Using a long-wave UV lamp, examine the colonies for fluorescence. Fluorescence indicates the presence of *E. coli*.

Make sure to examine the petri dish in a darkened area.

Some UV lamps do not use the correct wattage and can give false results. Be sure to use the UV lamps and replacement bulbs that are specified in *Consumables and replacement items*.



8. 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

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

- 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

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

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

m-Endo Broth for presumptive total coliforms

Required media and reagents

Description	Unit	Catalog number
m-Endo prepared agar plates	15/pkg	2811615
m-Endo Broth Ampules, plastic	50/pkg	2373550
m-Endo Broth, 100 mL glass bottle	1 each	2373542
m-Endo Broth PourRite™ Ampules, glass (for total coliform presumptive)	20/pkg	2373520
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	1	2087742
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
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, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Loop, inoculating, disposable	25/pkg	2749125
Microscope, compound	1	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 60 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ($^{5}/_{16}$ in.) ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Aspirator, water	each	213102
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 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
Incubator, portable, 12 VDC	each	2569900
Incubator, water bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, water bath, 240 VAC, 50/60 Hz	each	2616302
Isopropyl alcohol	500 mL	1445949
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

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

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	1	2087742

Required apparatus (continued)

Description	Unit	Catalog number
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Loop, inoculating, disposable	25/pkg	2749125

Optional media, reagents and apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Burner, Bunsen	each	2162700
Inoculating Needle, disposable	25/pkg	2748925
Lauryl Tryptose Broth Ampules, sterile (for enrichment technique)	20/pkg	1472520
Rack, coliform tube	each	221500
Wicks, replacement (used with Alcohol Burner 20877-42)	10/pkg	2097810

Confirmation of fecal coliforms (EC medium broth)

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
EC Medium Broth Tubes (for fecal coliform confirmation)	15/pkg	1410415

Required apparatus

Description	Unit	Catalog number
Forceps, stainless steel	1	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Inoculating Needle, disposable	25/pkg	2748925
Loop, inoculating, disposable	25/pkg	2749125
Swabs, cotton, sterile (for confirmation)	100/pkg	2554300

Optional media, reagents and apparatus

Description	Unit	Catalog number
Incubator, portable, 12 VDC	1	2569900
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with North American style plug	each	2281400
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with European style plug	each	2281402
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302
Inoculating Loop, nichrome wire, with handle	1	2112100
Rack, coliform tube	each	221500

E. coli confirmation with EC/MUG

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
EC Medium with MUG Broth Tubes (for *E. coli* confirmation)	15/pkg	2471515

Required apparatus

Description	Unit	Catalog number
Forceps, stainless steel	1	2141100
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	1	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	1	2184302
Swabs, cotton, sterile (for confirmation)	100/pkg	2554300

Optional media, reagents and apparatus

Description	Unit	Catalog number
Ecoli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	1	2569900
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Inoculating Needle, disposable	25/pkg	2748925
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with North American style plug	each	2281400
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with European style plug	each	2281402
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302
Inoculating Loop, nichrome wire, with handle	1	2112100
Lamp, long-wave, ultraviolet, portable, 4 watt	1	2415200
Loop, inoculating, disposable	25/pkg	2749125
Rack, coliform tube	each	221500

E. coli confirmation with nutrient agar

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
Nutrient Agar with MUG prepared plates	15/pkg	2182115
Nutrient Agar with MUG Tubes, 2 tests/tube (for *E. coli* confirmation)	6/pkg	2437306

Required apparatus

Description	Unit	Catalog number
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
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
Forceps, stainless steel	1	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	1	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	1	2184302

Optional media, reagents and apparatus

Description	Unit	Catalog number
Beaker, 600 mL	1	50052
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with North American style plug	each	2281400
Lamp, long-wave, ultraviolet, portable, 4 watt	1	2415200

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