

## m-ColiBlue24 Broth PourRite Ampules<sup>1</sup>

Method 10029

Membrane Filtration

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

<sup>1</sup> USEPA approved.



### 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  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F). Let the incubator temperature become stable, then add the 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.

#### Items to collect

Description	Quantity
Broth ampule, m-ColiBlue24	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 6 for order information.

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## 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

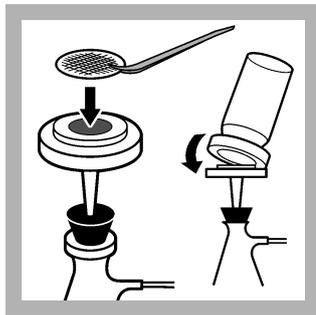
Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

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.

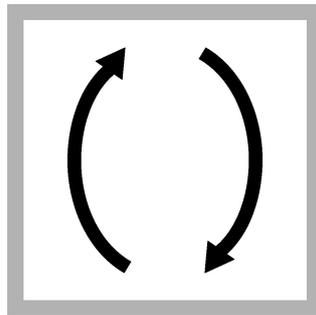
## Membrane filtration test procedure



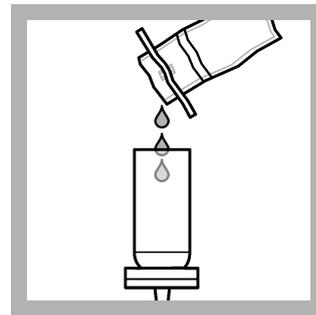
1. Invert one m-ColiBlue24 broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



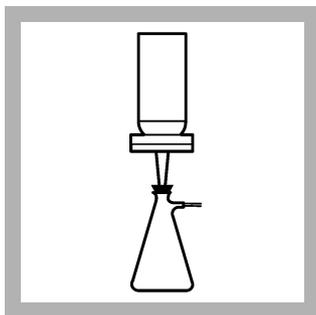
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



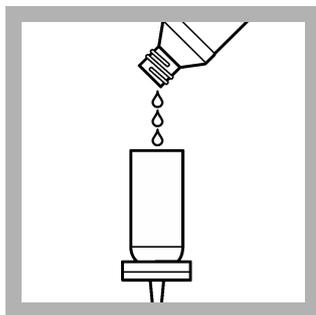
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



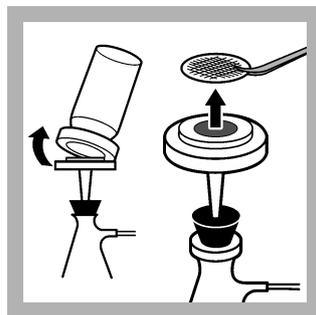
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



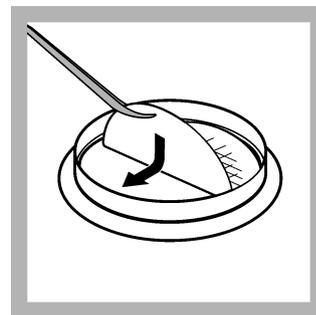
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



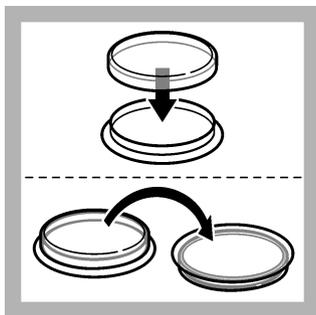
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



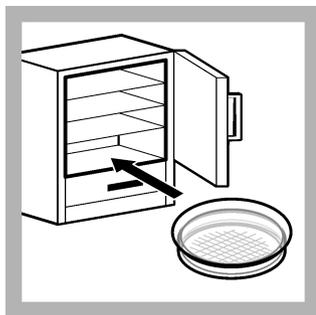
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



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  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for 24 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 5.

## Optional testing of red colonies

The m-ColiBlue24 Broth is made so that coliforms other than *E. coli* form red colonies. The percentage of red colonies that are false positives (non-coliforms) is equivalent to the

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percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms). So, a confirmation procedure is not necessary.

Some varieties of the non-coliform bacteria *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. can form on m-ColiBlue24 Broth and form red colonies. Use the oxidase method to quickly identify between these bacteria and total coliforms. *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If the sample has high levels of interfering bacteria, do an oxidase method to identify which of the red colonies are total coliforms.

Two oxidase methods follow. Count the red and blue colonies on the m-ColiBlue24 Broth membrane filter before the oxidase method is started.

### Oxidase method 1

Use this method to easily and quickly analyze membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth.

Research<sup>1</sup> shows that the oxidase method cannot be done on media where acidification of the media occurs during bacterial growth. The m-ColiBlue24 Broth is made so that acidification of the medium does not occur. As a result, the method can analyze many colonies at the same time for their oxidase reaction.

1. Remove the lid from the petri dish.
2. Invert the lid.
3. Put the lid on the bench top.
4. Use a dropper to put 0.5 mL of Difco SpotTest Oxidase Reagent in the center of the inverted lid.
5. Use sterile forceps to move the membrane filter from the pad in the petri dish to the inverted lid.

After 10 to 15 seconds, the filter absorbs the Difco SpotTest Oxidase Reagent. The oxidase-positive colonies change from red to purple. This purple color shows in the colony or adjacent to the colony. Oxidase-negative colonies stay red.

6. After the initial 10 to 15 second reaction time, count the red colonies that are purple in 30 seconds or less. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter.

For easy colony counting, put a spare lid on the petri dish lid. Use a felt-tip pen to put a mark on each purple colony. After 30 seconds, count the marks.

**Note:** The red oxidase-negative colonies start to change to a purple color after 30 seconds after the initial 10 to 15 second reaction time.

Bacteria are not killed with this method, so colonies can be selected for streaking and for more testing.

Colonies that are blue after the initial 24-hour incubation are almost always *E. coli*. So, confirmation with the oxidase method is not necessary.

### Oxidase method 2

This method is the official oxidase test in *Standard Methods for the Examination of Water and Wastewater*, 18th edition, 1992.

1. Select red colonies from an m-ColiBlue24 Broth membrane filter and streak on Tryptic Soy Agar.
2. Incubate the Tryptic Soy Agar plates at 35 °C (95 °F) for 18 to 24 hours or until the isolated colonies form.

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<sup>1</sup> A.H. Havelaar et al. 1980. *False-negative oxidase reaction as a result of medium acidification*. *Antonie van Leeuwenhoek*. 46, 301-312. L.K. Hunt et al. 1981. *Role of pH in oxidase variability of Aeromonas hydrophila*. *Journal of Clinical Microbiology*. 13: 1054-1059.

3. Soak a piece of filter paper with Difco SpotTest Oxidase Reagent. This reagent has a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.  
**Note:** As an alternative, use a dropper to add oxidase reagent directly on colonies on Tryptic Soy Agar. Oxidase-positive colonies change from pink to purple.
4. Use a sterile nichrome inoculating needle to move cellular material from an isolated Tryptic Soy Agar colony to the moist filter paper.  
**Note:** Do not use iron or other reactive needles for inoculation, because they cause false-positive results. Wooden applicator sticks work well.  
  
Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies cause the reagent to change to dark purple within 10 seconds.
5. Count the oxidase-negative colonies as total 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
<b>One membrane filter</b>	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 <math>50 \div 20 \text{ mL} \times 100 = 250</math> coliforms in 100 mL of sample.</i>
<b>Multiple filters, dilutions or duplicates for each sample</b>	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 <math>(5 + 9) \div (50 + 50) \times 100 = 14</math> 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

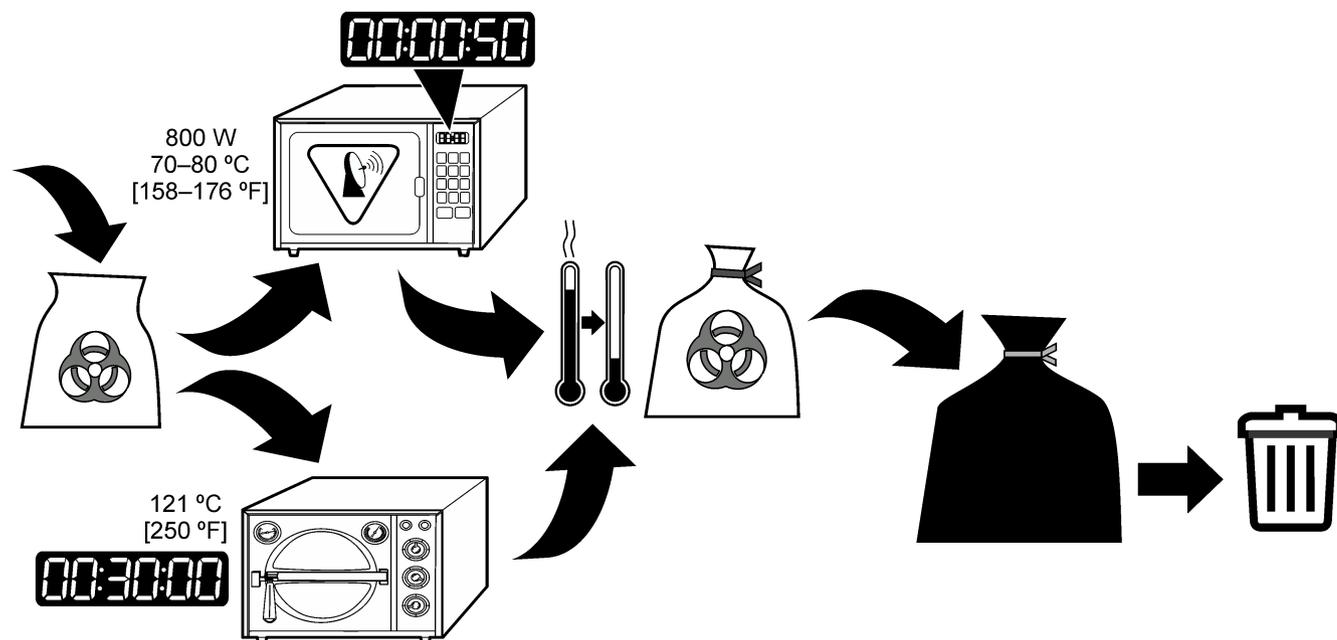
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive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

## 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

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages, surface water, well water, groundwater, waste water, recreational waters and process water for ultrapure, chemical processing and pharmaceutical applications.

Count all of the red and blue colonies as total coliforms. Count all of the blue colonies as *E. coli*. Blue colonies can be blue to purple.

**Note:** Sometimes only the center of a colony shows color. Count a colony with any red color as red. Count a colony with any blue as a blue colony. Red colonies can be different in color intensity.

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

### Required reagents

Description	Quantity/test	Unit	Item no.
m-ColiBlue24 <sup>®</sup> broth ampules, glass	1	20/pkg	2608420
m-ColiBlue24 <sup>®</sup> broth ampules, plastic	1	50/pkg	2608450

## Consumables and replacement items (continued)

Description	Quantity/test	Unit	Item no.
m-ColiBlue24 <sup>®</sup> , prepared agar plates	1	15/pkg	2805215
Dilution water, buffered, 99 mL, sterile <sup>2</sup>	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 <sup>®</sup> , 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 <sup>®</sup> 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

<sup>2</sup> Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

## Optional reagents and apparatus

Description	Unit	Item no.
m-ColiBlue24 <sup>®</sup> Broth, glass bottle	100 mL	2608442
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



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