Coliforms—Total and *E. coli*

DOC316.53.001213

USEPA Membrane Filtration Method **Method 10029[1]**

m-ColiBlue24®

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

[1] USEPA approved.

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.

m-ColiBlue24 Broth PourRite Ampules

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

Simultaneous total coliform and *E. coli* screening, method 10029

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

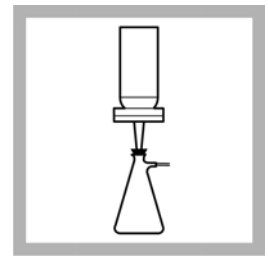
Do not touch the pad or the inside of the petri dish.

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

2. Invert ampules two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

3. Set up the Membrane Filter Apparatus. With sterile forceps, place a membrane filter, grid side up, into the assembly.

Alternatively, a sterile disposable filter unit may be used.

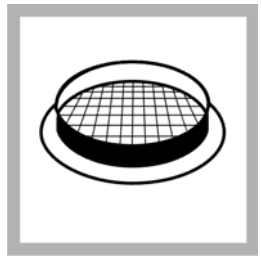


4. Invert the sample for 30 seconds to mix. Pour 100 mL of sample or diluted sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Rinse again two more times.

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

Simultaneous total coliform and *E. coli* screening, method 10029 (continued)

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



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air 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 24 hours.

8. Remove the petri dish from the incubator and examine the filters for colony growth. Colonies are typically readily visible; however, a microscope or other 10–15X magnifier may be useful. Red and blue colonies indicate total coliforms and blue colonies specifically indicate *E. coli*.

Sometimes only the center of a colony will show color. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as *E. coli.*

Optional testing of red colonies

The m-ColiBlue24 Broth is formulated so that coliforms other than *E. coli* grow as red colonies. The percentage of red colonies that are false positives (non-coliforms) is comparable to the percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms); therefore, confirmation is not required.

A few varieties of the non-coliform bacteria *Pseudomonas, Vibrio,* and *Aeromonas* spp. may grow on m-ColiBlue24 Broth and form red colonies. Such bacteria can be readily distinguished from total coliforms by the oxidase test. *Pseudomonas, Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If your sample contains high levels of interfering bacteria, you can perform an oxidase test to confirm which red colonies are total coliforms.

Two oxidase procedures are provided. Count the red and blue colonies on the m-ColiBlue24 Broth membrane filter before starting the oxidase test.

Oxidase method 1
This method enables you to conveniently and rapidly evaluate membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth.

Research* shows that the oxidase test cannot be performed on media that undergoes acidification during bacterial growth. The m-ColiBlue24 Broth is formulated so that the medium does not undergo such acidification. Consequently, many colonies can be simultaneously tested for their oxidase reaction using the following procedure.

1. Remove the lid from the petri dish containing the m-ColiBlue24 Broth membrane filter, invert the lid, and place it on the bench top.

   Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive controls and *Escherichia coli* for negative controls. Use Aqua QC-Stiks™ for quality control procedures.

2. Drop approximately 0.5 mL of Difco SpotTest™ Oxidase Reagent into the center of the inverted lid.

3. Using sterile forceps, transfer the membrane filter from the pad and place the filter upright in the inverted lid.

4. Within 10 to 15 seconds, the oxidase reagent will soak into the filter and cause the oxidase-positive colonies to turn purple. This purple color may be visible in the colony itself or adjacent to the colony. Oxidase-negative colonies will retain the red color they developed when incubated on m-ColiBlue24 Broth.

5. After the initial 10 to 15 second reaction time, start counting the red colonies that turn purple. Count individual colonies by using a microscope with 10–15X magnification

   Note: *To simplify colony counting place a spare lid on the lid containing the oxidase reagent and membrane filter. Use a felt-tip pen to mark the lid as you identify the purple colonies. After 30 seconds, you can count marks that indicate purple (oxidase-positive) colonies.*

6. Stop counting 30 seconds after initial 10 to 15 second reaction time, because oxidase-negative colonies will start to develop a purple color.

   Note: *Bacteria are not killed with this procedure, so colonies may be selected for streaking and for additional testing.*

Colonies that are blue after the initial 24-hour incubation on m-ColiBlue24 Broth are almost always *E. coli* and do not need confirmation with the oxidase procedure.

Oxidase method 2
This method is the official oxidase test described 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 onto Tryptic Soy Agar.

2. Incubate Tryptic Soy Agar plates at 35 °C (95 °F) for 18–24 hours or until isolated colonies are obtained.

* 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.

Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive and *Escherichia coli* for negative controls. Use Aqua QC-Stiks™* for quality control procedures.

3. Saturate a piece of filter paper with Difco SpotTest Oxidase Reagent. (This reagent contains a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.)

   Note: *Alternatively, oxidase reagent can be dropped directly onto colonies growing on Tryptic Soy Agar. Oxidase-positive colonies will turn from pink to purple.*

4. Using a sterile nichrome inoculating needle, transfer 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 will cause false-positive results. Wooden applicator sticks work well.*

5. Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies will cause the reagent to turn dark purple within 10 seconds.

6. Oxidase-negative colonies should be counted as total coliform bacteria.

Interpreting and reporting results

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

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

Equation A—Coliform density on a single membrane filter

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

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

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

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

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

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

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

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

* Aqua QC-Stiks is a trademark of MicroBiologics.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-ColiBlue24® Broth Ampules, glass	20/pkg	2608420
m-ColiBlue24® Broth Ampules, plastic	50/pkg	2608450
m-ColiBlue24® prepared agar plates	15/pkg	2805215

Required apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598
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)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	each	2486100
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Microscope, Compound	each	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ($^{5}/_{16}$ 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
Alcohol Burner	1	2087742
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
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

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
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
Bunsen burner with tubing	each	2162700
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Incubator, water bath, 120 VAC, 50/60 Hz	each	2616300
Isopropyl alcohol	500 mL	1445949
m-ColiBlue24® Broth, 100 mL glass bottle	1 each	2608442
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
Wicks, replacement, for alcohol burner 2087742	—	2097810

[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.

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