

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

¹ USEPA accepted.

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.

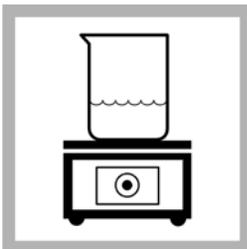
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.

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-TEC Agar confirmation for presumptive *E. coli* test

The m-TEC method detects *E. coli* in recreational fresh water samples with a two step process. First, membrane filters are incubated on m-TEC Agar for 2 hours at 35 °C to resuscitate injured organisms. The thermotolerant organisms are then selected by fermentation of lactose at an elevated temperature of 44.5 °C. The second step uses a substrate medium containing urea to distinguish urease-negative *E. coli* from other thermotolerant coliforms that hydrolyze urea. Yellow or yellow-brown urease-negative colonies are positive for *E. coli*.

Presumptive *E. coli* test (m-TEC Agar), method 8367



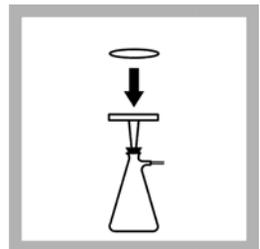
1. Heat a beaker of water or water bath but do not allow it to boil.



2. Place m-TEC Agar tubes into hot water. When agar melts, carefully remove tubes from hot water with tongs.

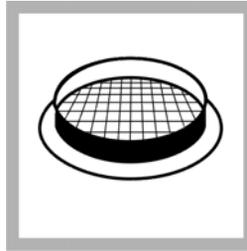
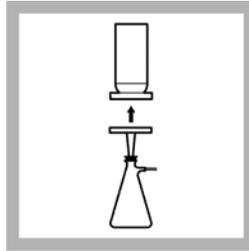
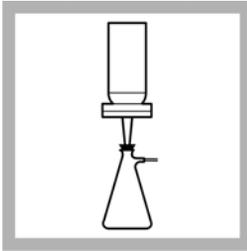


3. Using sterile technique, pour half of the contents of the tube into a sterile, 47-mm petri dish. Immediately replace the petri dish lid and allow agar to solidify undisturbed.



4. Set up the Membrane Filter Assembly. Using sterilized forceps, place a membrane filter, grid side up, into the assembly. To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

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5. Prepare the necessary dilutions to obtain the proper sample size. Invert the sample for 30 seconds to mix. Pour 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. Repeat the rinsing step, two more times.

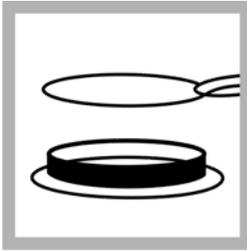
Release the vacuum when the filter is dry to prevent damage to the filter.

6. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the previously prepared petri dish.

7. With a slight rolling motion, place 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.

8. Invert the petri dish. Incubate at 35 ± 0.5 °C for 2 hours and then at 44.5 ± 0.2 °C for 22 hours.

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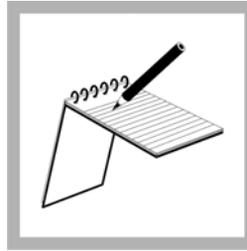
9. To confirm, use sterilized forceps to transfer the filter to a pad saturated with at least 2 mL of urea substrate.

Preparing the urea substrate:

- a. Dissolve 2.0 g of urea in 100 mL of deionized water.
- b. Add 10 mg of phenol red sodium indicator to the urea solution.
- c. Use 0.02 N sulfuric acid to adjust the pH to between 3 and 4. The solution will turn yellow.
- d. Store solution at 2 to 8 °C. Use within one week.



10. After 15 minutes, count yellow or yellow-brown colonies by using a 10 to 15X microscope. The presence of yellow or yellow-brown colonies confirms *E. coli*.



11. 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

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

- 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.

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

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-TEC Agar Tubes, 2 tests/tube (for <i>E. coli</i> determination)	6/pkg	2561106
Phenol Red Sodium Salt	5 g	2563922
Sulfuric Acid, 0.02 N	100 mL	20342
Urea Reagent, ACS	100 g	1123726

Required apparatus

Description	Unit	Catalog number
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
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 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
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
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.



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

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