

# Coliforms—Total, Fecal and *E. Coli* DOC316.53.01216

USEPA<sup>1</sup> Lauryl Tryptose Broth presumptive test with BGB, EC Medium and EC/MUG confirmation

Method 8001

## Most Probable Number (MPN) Method

**Scope and Application:** For potable water.

<sup>1</sup> Most Probable Number Method 8001 for potable water is USEPA-accepted. Method 8001 meets or exceeds the specification criteria stated in Standard Methods for the Examination of Water and Wastewater, 19th edition, 9221 Multiple-Tube Fermentation Technique for Members of the Coliform Group. For potable water, confirm fecal coliforms with EC Medium Broth as cited in 40 CFR Part 141.21, Subpart (F)(5); or confirm *E. coli* with EC/MUG Medium Broth as cited in 40 CFR Part 141.21, Subpart (F)(6)(i).

### Before starting the test:

Make sure that all materials that are used for containing or transferring samples are sterile.

Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

Use the Lauryl Tryptose Broth for the presumptive test. If the presumptive test is positive, use Brilliant Green Bile (BGB) broth to confirm if the sample has total coliforms, then EC Medium for fecal coliforms or EC Medium with MUG for *E. coli*.

Potable water should not contain any coliform bacteria. Samples should not be diluted.

For USEPA reporting, the confirmation tubes must be inoculated by an inoculation loop. Cap transfer is not allowed.

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

If all 10 tubes (for a 10-tube MPN test) of the confirmed coliform test are negative, the sample is accepted as meeting bacterial standards. To make sure that sample results are interpreted in accordance with appropriate standards and regulations, contact the local, county, state or federal regulatory agency.

If the test will not be used for USEPA reporting, 5 broth tubes can be used in place of 10 tubes. Use the [MPN table for 5 tubes](#) to find the result of the 5-tube test. The 5-tube test cannot be used for USEPA reporting.

### Collect the following items:

Description	Quantity
Lauryl Tryptose Broth Tubes	10
Brilliant Green Bile (BGB) Broth Tubes	varies
EC Medium Broth Tubes	varies
EC Medium with MUG Broth Tubes	varies
Incubator	1
Alcohol burner	1
Inoculating loop	1
Pipet, serological, 10–11 mL, sterile	1
Pipet filler	1
Coliform tube rack	1

See [Consumables and replacement items](#) for reorder information.

Presumptive test for coliform bacteria (Lauryl Tryptose Broth)



1. Wash thoroughly with soap and water. Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed.



2. Remove the caps from 10 tubes of Lauryl Tryptose Broth one at a time. Use a sterile pipet to transfer 10 mL of sample into each of the tubes. Do not touch the open end of the tubes or the inside of the caps.



3. Replace and tighten the screw cap on each tube immediately after the sample is added. Invert and swirl the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure the inner vial is full of liquid with no air bubbles.



4. Place the tubes in the incubator at a temperature of 35 (±0.5) °C.



5. After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator. Continue incubation. The tubes must be kept upright for the rest of the test.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure there are no bubbles and then carefully return the tubes to an upright position.



6. After 24 (±2) hours, 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 present. If no gas can be seen, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

The presence of gas in any amount is an indication of coliform bacteria.

Count the number of tubes that contain gas in the inner vial.

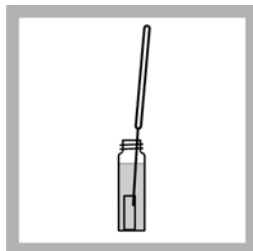


7. Complete a confirmation test for all tubes that contain gas. The confirmation test will confirm whether total coliforms, fecal coliforms or E.Coli are present in the sample.

The confirmation test is used to eliminate false-positive results that can occur with the presumptive test.

If none of the tubes contain gas, the test is negative for coliform bacteria.

## Confirmation test for total coliforms (Brilliant Green Bile Broth)



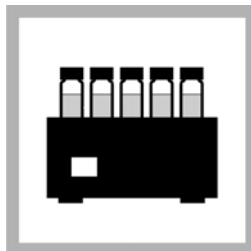
1. From each positive LT Broth tube, inoculate a Brilliant Green Bile (BGB) Broth tube. Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a BGB Broth tube, making sure not to touch the rim of either tube.



2. Replace and tighten the screw cap on each tube immediately. Invert and swirl the BGB tubes to remove trapped air in the inner vials.

If Durham tube vials are used, after the last inversion, make sure that the inner tube does not contain any air bubbles.



3. Place the BGB tubes in the incubator at a temperature of  $35 (\pm 0.5) ^\circ\text{C}$ .



4. After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Continue incubation. The tubes must be kept upright for the rest of the test.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure the bubbles are gone and then carefully return the tubes to an upright position.



5. After 24 ( $\pm 2$ ) hours, tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria.

If no gas can be seen, return the tubes to the incubator and examine again after a total of 48 ( $\pm 3$ ) hours.



6. After 48 ( $\pm 3$ ) hours, tap each tube gently and examine the inner vials for gas. If the inner vials contain gas bubbles, the test is positive for total coliform bacteria.

If none of the tubes contain gas, the test is negative for coliform bacteria.



7. Count the number of tubes that contain gas in the inner vial.

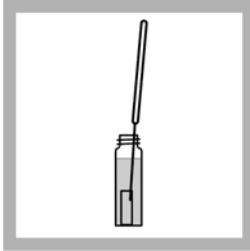
Find the MPN of the sample (total coliform bacteria per 100 mL sample) from the [MPN table for 10 tubes](#).



8. If the test is positive for total coliform bacteria, complete a confirmation test for fecal coliform or E. Coli bacteria (USEPA requirement).

**Confirmation test for fecal coliforms (EC Medium)**

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1. From each positive LT Broth tube, inoculate an EC Medium Broth tube.

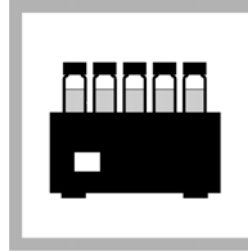
Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a EC Medium tube, making sure not to touch the rim of either tube.



2. Replace and tighten the screw cap on each tube immediately. Invert and swirl the EC Medium Broth tubes to remove trapped air in the inner vials.

If Durham tube vials are used, after the last inversion, make sure that the inner tube does not contain any air bubbles.



3. Place the EC Medium Broth tubes in the incubator at a temperature of 44.5 (±0.2) °C.



4. After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Continue incubation. The tubes must be kept upright for the rest of the test.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure the bubbles are gone and then carefully return the tubes to an upright position.



5. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for fecal coliform bacteria.

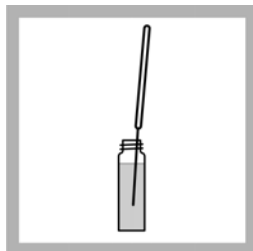
If none of the tubes contain gas, the test is negative for fecal coliform bacteria.



6. Count the number of tubes that contain gas in the inner vial.

Find the MPN of the sample (fecal coliform bacteria per 100 mL sample) from the [MPN table for 10 tubes](#).

## Confirmation test for *E. coli* (EC Medium with MUG)



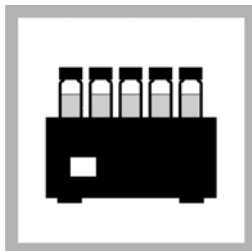
1. From each positive LT Broth tube, inoculate an EC Medium with MUG Broth tube.

Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

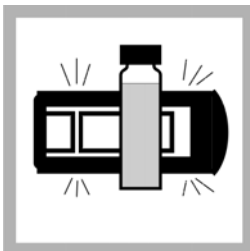
Put the loop into the positive Lauryl Tryptose tube and then into a EC Medium with MUG tube, making sure not to touch the rim of either tube.

Replace and tighten the screw cap on each tube immediately. Invert and swirl to mix.

If Durham tube vials are used, after the last inversion, make sure that the inner tube does not contain any air bubbles.



2. Place the EC Medium with MUG tubes in the incubator at a temperature of 44.5 ( $\pm 0.2$ ) °C for 24 ( $\pm 2$ ) hours.



3. After 24 ( $\pm 2$ ) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

Compare the fluorescence of the sample tubes to a tube containing a known *E. coli* culture to make a positive confirmation.

If the solution shows fluorescence, the test is positive for *E. coli*. If there is no fluorescence, the test is negative for *E. coli*.



4. Count the number of tubes that show fluorescence.

Find the MPN of the sample (*E. coli* bacteria per 100 mL sample) from the [MPN table for 10 tubes](#).

## Sample collection, preservation and storage

- Collect at least 100 mL of sample in sterilized Whirl-Pak® bags, sterilized disposable bottles or autoclaved glass or plastic bottles.
- Do not fill sample containers completely. Leave at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.
- Make sure that the samples are representative of the sample source. Fill sample containers from a tank or reservoir entirely under water.
- Start the analysis as soon as possible after collection. Allow no more than 30 hours to elapse after collection. If the test cannot be started immediately, cool the sample to below 10 °C. Do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

## MPN table

Use the number of positive tubes to find the MPN per 100 mL from the [MPN table for 10 tubes](#).

**Example:** 6 of the 10 tubes showed a positive response. The MPN per 100 mL is 9.2.

**Table 432 MPN table for 10 tubes<sup>1</sup>**

Number of positive tubes	MPN per 100 mL
0	< 1.1
1	1.1
2	2.2
3	3.6
4	5.1
5	6.9
6	9.2
7	12.0
8	16.1
9	23.0
10	> 23.0

<sup>1</sup> Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits.

If the test will not be used for USEPA reporting, 5 broth tubes can be used in place of 10 tubes and the [MPN table for 5 tubes](#) can be used. The 5-tube test cannot be used for USEPA reporting.

**Table 433 MPN table for 5 tubes<sup>1</sup>**

Number of positive tubes	MPN per 100 mL
0	< 2.2
1	2.2
2	5.1
3	9.2
4	16.0
5	> 16.0

<sup>1</sup> Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits. The MPN table for 5 tubes cannot be used for USEPA reporting.

## Bacteria disposal

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

### Bleach

Sterilize used test tubes with household bleach. Add 1–2 mL of the bleach to each test tube. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

### Autoclave

Place used test tubes in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test tubes in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag and tie tightly.

## Summary of method

The Most Probable Number (MPN) method (also referred to as the Multiple Tube Fermentation Technique) uses screw-capped tubes containing sterile broth medium. The tubes contain an inverted inner vial (Durham tube) for gas collection. Sample is added to the tubes and incubated. If coliforms are present, gas is produced and is trapped in the inner vial. The number of tubes that form gas is used to estimate the number of coliform organisms in the sample. When the EC Medium with MUG Broth is used, fluorescence under a longwave UV lamp confirms the presence of *E. coli*.

## Consumables and replacement items

### Required media and reagents

Description	Unit	Catalog number
Lauryl Tryptose Broth tubes	15/pkg	2101415
Brilliant Green Bile (BGB) Broth tubes	15/pkg	32215
EC Medium Broth tubes	15/pkg	1410415
EC Medium with MUG Broth tubes (without Durham tubes)	15/pkg	2471515
EC Medium with MUG Broth tubes (with Durham tubes)	15/pkg	2282415

### Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Bags, Whirl-Pak®, with dechlorinating agent, 170-mL	100/pkg	2075333
Incubator, 12-well Dri-Bath, 120 VAC, 50/60 Hz	each	2281400
Inoculating Loop, nichrome wire	each	2112100
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	each	2184302
Pipet, serological, 10-11 mL, sterile, disposable	25/pkg	209798
Pipet safety bulb	each	1465100

### Optional media and reagents

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dilution Water, buffered, 99-mL, sterile	25/pkg	1430598
Powder Pillows for buffered dilution water (25 of each) <sup>1</sup>	50/pkg	2143166

<sup>1</sup> 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.

## Coliforms—Total, Fecal and E. Coli

### Optional apparatus

Description	Unit	Catalog number
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	500/pkg	2233100
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700
E. coli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Inoculating Loops, sterile, disposable	25/pkg	2749125
Isopropyl alcohol	500 mL	1445949
Lamp, long-wave, ultraviolet, portable, 4 watt	each	2415200
Marker, laboratory	each	2092000
Pipet, serological, 1-mL, sterile, disposable, individually wrapped	50/pkg	2092835
Pipet, serological, 10-mL, sterile, disposable, individually wrapped	50/pkg	2092628
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, sterile, individually wrapped	200/pkg	2558996
Pipet Aid, 110 VAC recharger, 4 replacement filters (UL, CSA approved)	each	2551701
Rack, coliform tube	each	221500
Wicks, replacement, for alcohol burner 2087742		2097810



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