Coliforms—Total and *E. Coli*

DOC316.53.01218

Lauryl Tryptose with MUG Broth[1]

Method 8091

Most Probable Number (MPN) Method

Scope and Application: For potable and non-potable water.

[1] Based on publication by Peter C.S. Feng and Paul A. Hartman "Fluorogenic Assays for Immediate Confirmation of Escherichia coli". *Applied and Environmental Microbiology*, Vol. 43, No. 6, pp. 1320-1329, 1982. This method is not USEPA accepted.

Before starting the test:

Make sure that all materials used for containing or transferring samples are sterile.
The bottles of dilution water contain 99 mL of sterile buffered dilution water. When 11 mL of sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10-fold or 10x dilution). Be sure to mix the bottles thoroughly before and after the sample is added.
If all the tubes are positive, dilute the sample several more times and repeat the test. If all tubes are negative, the sample was diluted too many times. Repeat the test with less serial dilutions.
If more than three dilutions were made, use three consecutive dilutions that contain both positive and negative tubes.
The dilution factor for an undiluted sample is 1.
Fluorescence without gas production indicates an anaerogenic (non-gas-producing) strain(s) of *E. coli*.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray, or dilute iodine solution. Wash hands thoroughly with soap and water.

Collect the following items:

Description	Quantity
Lauryl Tryptose with MUG Broth Tubes	5–15
Dilution Water, buffered, 99-mL, sterile (for non-potable water samples only)	3 bottles
Coliform tube rack	1
Incubator	1
Pipet, serological, 10-11 mL, sterile	3
Pipet filler	1

See *Consumables and replacement items* for reorder information.

Potable water test for coliforms—total and *E. coli*, Lauryl Tryptose with MUG 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 5 or 10 tubes of Lauryl Tryptose with MUG 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 the screw cap on each tube immediately after the sample is added. Invert 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.

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.

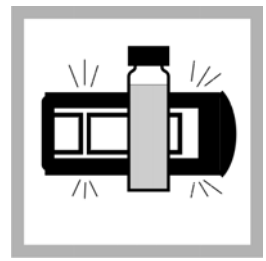
Loosen the caps slightly before returning the tubes to the incubator. Continue incubation. The tubes must be kept upright for the rest of the test.

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.

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

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

If tubes are cloudy but have no gas bubbles, check the tubes for fluorescence (step 7).

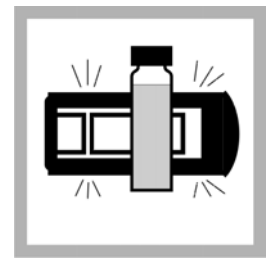


7. Use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If the tube does not fluoresce, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

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



8. After 48 (±3) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

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

Refer to *Potable water MPN results* to find the MPN of the sample.

Non-potable water test for coliforms—total and *E. coli*, Lauryl Tryptose with MUG Broth

1. Wash thoroughly with soap and water.

Invert the sample for 30 seconds, approximately 25 times, to make sure that it is well-mixed.

2. Using sterile buffered dilution water, prepare at least 3 serial dilutions of the sample.

Refer to *Sample dilution* for instructions.

3. Remove the caps from 15 tubes of Lauryl Tryptose with MUG Broth. Use a sterile pipet to transfer 10 mL of the first dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the second dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the third dilution into the remaining 5 tubes.

Do not touch the open end of the tubes or the inside of the caps.

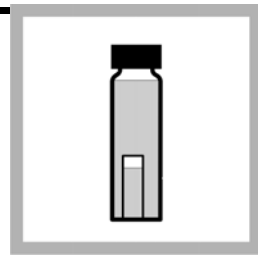
4. Replace the screw cap on each tube immediately after the sample is added. Invert the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure the inner vial is full of liquid with no air bubbles.

Non-potable water test for coliforms—total and *E. coli*, Lauryl Tryptose with MUG Broth (continued)

5. Place the tubes in the incubator at a temperature of 35 ±0.5 °C.

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.

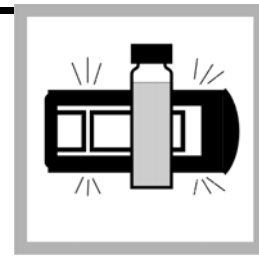


6. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely present.

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

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

If tubes are cloudy but have no gas bubbles, check the tubes for fluorescence (step 7).

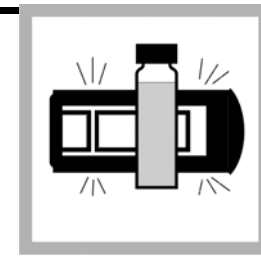


7. Use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If the tube does not fluoresce, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

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



8. After 48 (±3) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If there is no fluorescence, the test is negative for E. coli.

Refer to *Non-potable water MPN results* to find the MPN of the sample.

Sample dilution

Complete the following procedure to make serial dilutions of the sample. Refer to the *Dilution guidelines by sample type* to find the number of times the sample must be diluted. Use the three dilutions from the *Dilution guidelines by sample type* for the test.

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container for 30 seconds, approximately 25 times.
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 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until there are three bottles that contain the dilutions listed in the *Dilution guidelines by sample type*.

Note: *Shaking the sample too vigorously will injure or stress the organisms.*

Table 426 Dilution guidelines by sample type

Sample type	Dilution 1	Dilution 2	Dilution 3
Swimming pool water, chlorinated	undiluted (1x)	10x	100x
Bathing beach water	10x	100x	1000x
Lake water	10x	100x	1000x
Unpolluted river water	10x	100x	1000x
Final effluent, chlorinated	100x	1000x	10,000x
River water, polluted	1000x	10,000x	100,000x
Storm water	10,000x	100,000x	1,000,000x
Unchlorinated final effluent	10,000x	100,000x	1,000,000x
Raw sewage	100,000x	1,000,000x	10,000,000x

Potable water MPN results

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 427 MPN table for 10 tubes[1]

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

[1] Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits.

5 broth tubes can be used in place of 10 tubes and the *MPN table for 5 tubes* can be used.

Table 428 MPN table for 5 tubes[1]

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

[1] Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits.

Non-potable water MPN results

A sample was diluted into 3 different buffered dilution bottles. The dilutions were 10-fold (10x), 100-fold (100x) and 1000-fold (1000x). 5 MPN tubes were filled from each dilution (15 tubes total). The first set of tubes (10x) had four tubes with gas, the second set (100x) had 2 tubes with gas and the third set (1000x) had 1 tube with gas.

1. Find the MPN index for the three sets of tubes from the *MPN index table*.
2. Multiply the MPN index by the lowest dilution factor.

The MPN index from the *MPN index table* table for 4, 2 and 1 positive tubes is 26. The coliform result for the sample is 26 x 10 = 260 coliforms per 100 mL of sample.

Table 429 MPN index table

Number of positive tubes			MPN index per 100 mL	Number of positive tubes			MPN index per 100 mL
First dilution set	Second dilution set	Third dilution set		First dilution set	Second dilution set	Third dilution set	
0	0	0	< 2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
2	0	0	4	5	1	2	60
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
4	0	0	13	5	4	4	350
4	0	1	17	5	5	0	240
4	1	0	17	5	5	1	300
4	1	1	21	5	5	2	500
4	1	1	26	5	5	3	900
4	2	0	22	5	5	4	1600
				5	5	5	≥ 1600

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 6 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.

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.

Bacteria disposal

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

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

Autoclave
Place used test containers in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test containers 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 diluted, 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. The MPN method allows for the analysis of highly turbid samples by dilution prior to analysis. No filtering is necessary.

The Lauryl Tryptose with MUG Broth will detect total coliforms and *E. coli*. The results are comparable to the traditional MPN fecal coliform tests, but obtained in far less time. No transfer from presumptive to confirmed medium is necessary with the LT/MUG method. The LT/MUG medium contains lauryl tryptose broth and 4-methylumbelliferyl-ß-D-glucuronide (MUG), a fluorogenic reagent. Tubes positive for *E. coli* will fluoresce when the incubated tubes are examined under long-wave UV light.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Lauryl Tryptose with MUG Broth Tubes	15/pkg	2182115
Dilution Water, buffered, 99-mL, sterile	25/pkg	1430598

Required apparatus

Description	Unit	Catalog number
Bags, Whirl-Pak®, with dechlorinating agent, 180-mL	100/pkg	2075333
Incubator, 12-well Dri-Bath, 120 VAC, 50/60 Hz	each	2281400
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	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 reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 720-mL	100/pkg	1437297
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	500/pkg	2233100
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Brilliant Green Bile (BGB) Broth Tubes	15/pkg	32215
Bottle, polysulfone, autoclavable (use for buffered dilution water)	12/pkg	2245300
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottle, sample, sterilized, 100-mL fill-to line, disposable w/dechlorinating agent	50/pkg	2599150
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Ecoli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Incubator, Portable, 12 VDC	each	2569900
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

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Rack, Coliform Tube	each	221500
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199

[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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