

## Lauryl Tryptose with MUG Broth<sup>1</sup>

Method 8091

### Most Probable Number (MPN) Method

**Scope and application:** For potable and non-potable water.

<sup>1</sup> 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 accepted by the USEPA.



## Test preparation

### Before starting

Wash hands thoroughly with soap and water.

Make sure that all of the materials that come in contact with samples are sterile.

Use a dilute bleach solution, bactericidal spray or dilute iodine solution to clean the work area.

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.

If all tubes are positive, dilute the sample several times then do the test again. Do this until the dilution series gives both positive and negative tubes. If all of the tubes are negative, the sample was diluted too many times. Do the test again with less serial dilutions.

If more than three dilutions are made, select the three dilutions that are the most equivalent to the sample.

The dilution factor for an undiluted sample is 1.

The bottles of dilution water contain 99 mL of sterile buffered dilution water. When 11 mL of the sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10x or 10-fold dilution). Before and after the sample is added, make sure to fully mix the bottles.

Fluorescence without gas formation is an indication of an anaerogenic (non-gas-forming) strain(s) of *E. coli*.

Read the sections on [Sample collection](#) on page 2 and [Sample dilution](#) on page 6.

Refer to [Bacteria disposal](#) on page 9 for instructions on correct bacteria disposal.

### Items to collect

Description	Quantity
Lauryl Tryptose with MUG broth tubes	5–15
Dilution water, buffered, 99-mL, sterile	3 bottles
Incubator	1
Pipet, serological, 10–11 mL, sterile	1
Pipet filler bulb	3
MPN tube incubator rack	1

Refer to [Consumables and replacement parts](#) on page 10 for order information.

## Sample collection

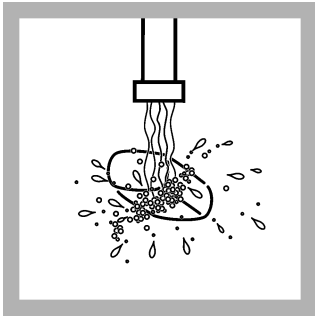
- Use a sterile glass or plastic container such as a Whirl-Pak® bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. 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 to 3 minutes. Remove any screens or aerators. Do not use faucets or spigots that swivel or leak.
- To collect a non-potable sample from a river, lake or reservoir, remove the cap under water. As an alternative, remove the cap and push the container, mouth down, into the water to prevent the collection of surface scum. Fill the container entirely under water. Put the mouth of the container into the current. Put the cap back on the container.
- Collect a minimum of 100 mL of sample and 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 the analysis cannot be started immediately, keep the sample at or below 10 °C (50 °F) for up to 6 hours. Do not let the sample freeze.
- Failure to collect and transport samples correctly will cause inaccurate results.

## Potable water test for coliforms—total and *E. coli*

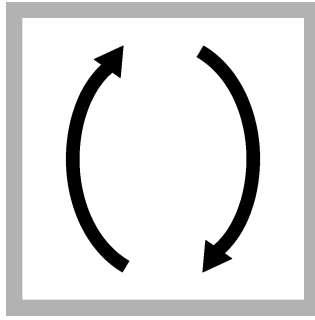
<b>⚠ CAUTION</b>	
	Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

When the nutritional media contains MUG, use a long-wave (e.g., 365 nm) UV lamp to confirm the presence of *E. coli*. The sample will fluoresce if *E. coli* is in the sample. No additional confirmation procedure is necessary.

**Note:** *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



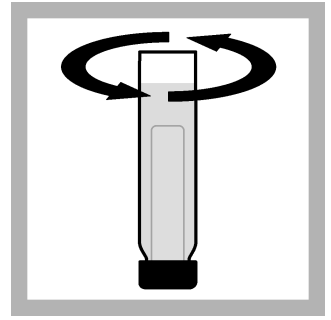
1. Wash hands thoroughly with soap and water.



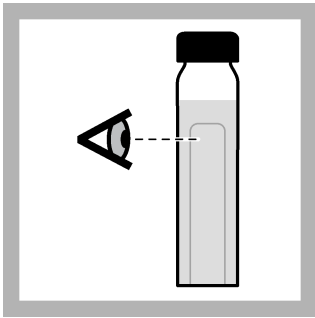
2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.



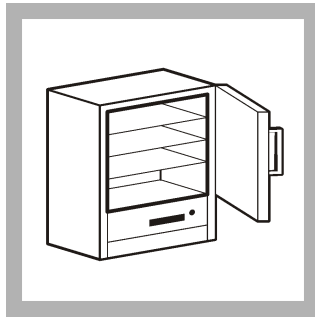
3. Remove the caps from five or 10 tubes of Lauryl Tryptose with MUG broth, one at a time. Use a sterile pipet to transfer 10 mL of sample into each tube. Do not touch the open end of the tubes or the inner surface of the caps. Immediately replace and tighten the screw cap on each tube.



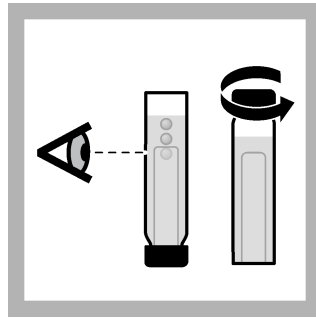
4. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



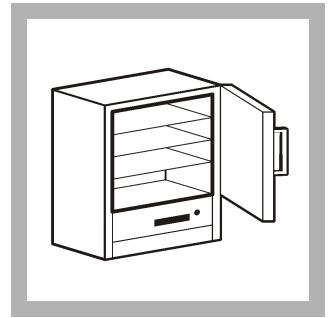
5. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



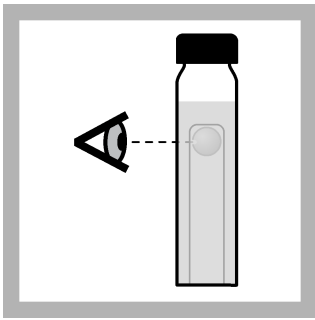
6. Incubate the sample at  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



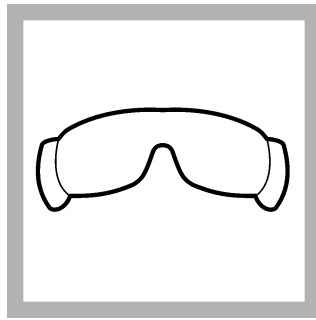
7. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



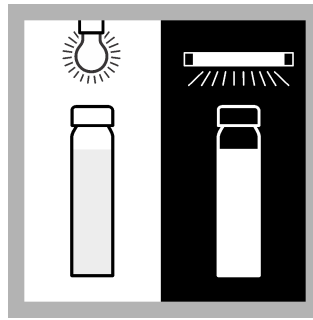
8. Incubate the sample at  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for 24 hours  
**Note:** *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



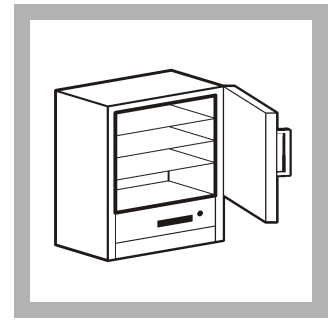
**9.** After  $24 \pm 2$  hours, remove the tubes from the incubator. Tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Gas in the inner vial is an indication of coliform bacteria. If the tubes are cloudy but have no gas bubbles, examine the samples for fluorescence. If no gas can be seen, the test is negative for total coliform bacteria.



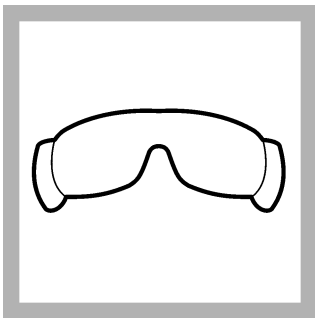
**10.** Put on UV safety goggles



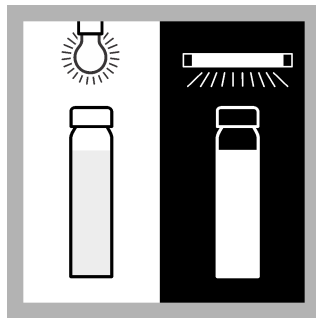
**11.** Apply UV light to the incubated samples with a long-wave UV lamp. Examine the tubes in a dark area. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* culture for a positive confirmation. If the sample fluoresces, the test is positive for *E. coli*. If the sample does not fluoresce, put the tubes back in the incubator for  $24 \pm 2$  hours.



**12.** Incubate the sample at  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for 24 hours (for a total of  $48 \pm 3$  hours) and examine the tubes again.



**13.** Put on UV safety goggles



**14.** After  $24 \pm 2$  hours (for a total of  $48 \pm 3$  hours), apply UV light to the incubated samples. Examine the tubes in a dark area. If the sample fluoresces, the test is positive for *E. coli*. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* culture for a positive confirmation. If there is no fluorescence, the test is negative for *E. coli*. Refer to [MPN results](#) on page 7 to find the MPN of the sample.

## Non-potable water test for coliforms—total and *E. coli*

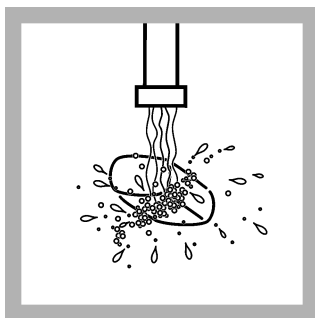
### ⚠ CAUTION



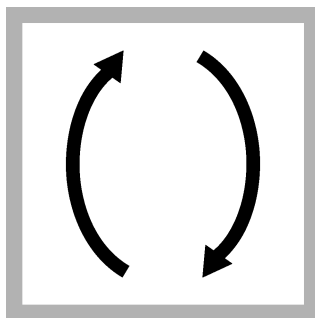
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

When the nutritional media contains MUG, use a long-wave (e.g., 365 nm) UV lamp to confirm the presence of *E. coli*. The sample will fluoresce if *E. coli* is in the sample. No additional confirmation procedure is necessary.

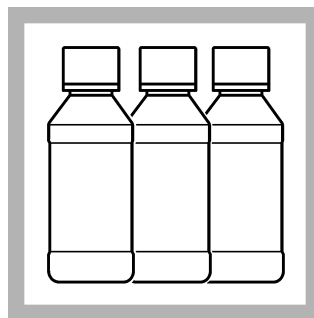
**Note:** The sample container can fluoresce slightly. To help with fluorescence detection, use an *E. coli* Fluorescence Standard. Compare the fluorescence from the sample and the standard.



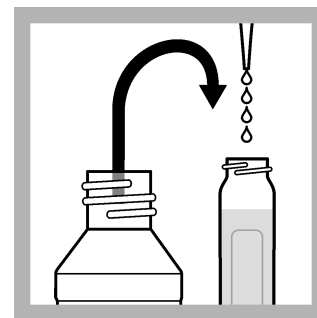
1. Wash hands thoroughly with soap and water.



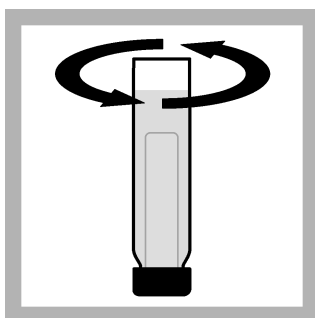
2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.



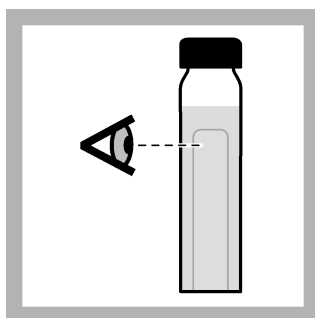
3. Prepare a minimum of three serial dilutions of the sample with sterile buffered dilution water.



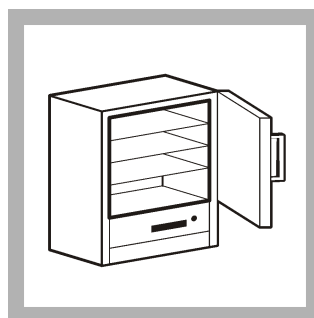
4. Remove the caps from 15 tubes of Lauryl Tryptose with MUG broth, one at a time. Use a sterile pipet to add 10-mL portions of each sample dilution into five tubes for the first dilution. Do this two more times for the second and third dilutions. Do not touch the open end of the tubes or the inner surface of the caps. Immediately replace and tighten the screw cap on each tube.



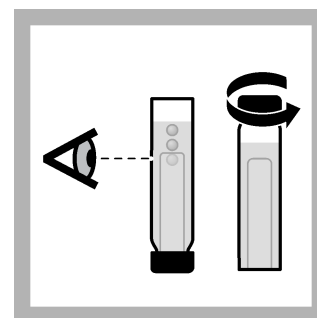
5. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



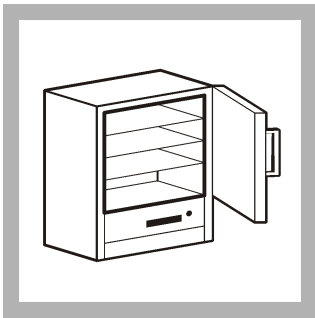
6. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



7. Incubate the sample at  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.

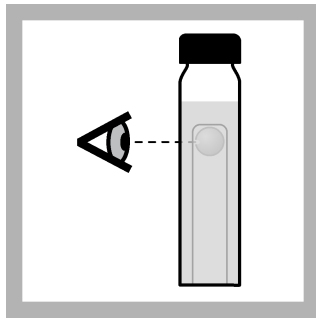


8. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



9. Incubate the inoculated confirmation media at  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for  $24 \pm 2$  hours.

**Note:** It is necessary to keep the tubes in a vertical position for the remainder of the test.

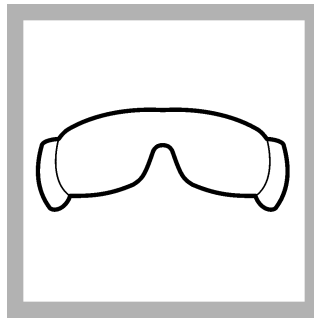


10. After  $24 \pm 2$  hours, tap each tube gently and examine the inner vials for gas.

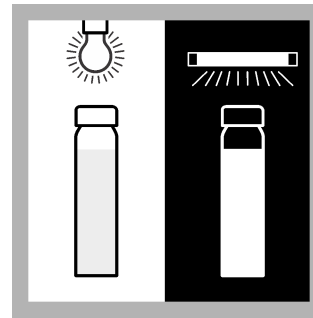
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Gas in the inner vial is an indication of coliform bacteria.

If the broth is cloudy but the inner vials do not contain gas bubbles, examine the samples for fluorescence.

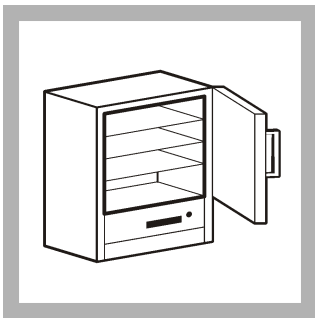
If none of the tubes contain gas, the test is negative for total coliform bacteria.



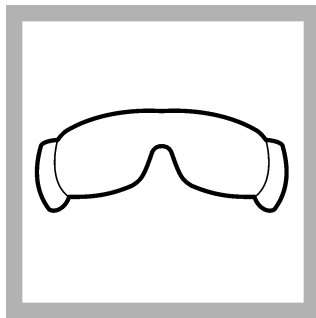
11. Put on UV safety goggles



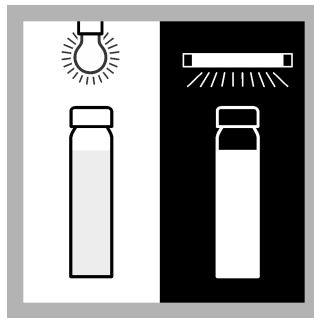
12. Apply UV light to the incubated samples with a long-wave UV lamp. Examine the tubes in a dark area. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* culture for a positive confirmation. If the sample fluoresces, the test is positive for *E. coli*. If the sample does not fluoresce, put the tubes back in the incubator for  $24 \pm 2$  hours.



13. Incubate the sample at  $35 \pm 0.5$  °C ( $95 \pm 0.9$  °F) for an additional  $24 \pm 2$  hours.



14. After  $24 \pm 2$  hours (for a total of  $48 \pm 3$  hours), remove the sample from the incubator. Put on UV safety goggles.



15. Apply UV light to the incubated samples. Examine the tubes in a dark area. If the sample fluoresces, the test is positive for *E. coli*. If there is no fluorescence, the test is negative for *E. coli*. Refer to [Example calculation](#) on page 8 to find the MPN of the sample.

## Sample dilution

Do the steps that follow to make serial dilutions of the sample.

**Example:** For Class A sludge, add 10 mL of the 100x sample dilution into five tubes, 10 mL of the 1000x sample dilution into another five tubes and 10 mL of the 10,000x sample dilution into the last five tubes. If the coliform density is not known, add five separate dilutions to five sets of five MPN tubes.

1. Wash hands thoroughly with soap and water. Gloves are optional.
2. Vigorously mix the sample for 30 seconds.
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 10-fold 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. Continue to make dilutions until there are three bottles that contain the dilutions listed in [Table 1](#).

**Note:** Do not vigorously shake the sample because this will injure or stress the organisms.

**Table 1 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 wastewater 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 wastewater effluent	10,000x	100,000x	1,000,000x
Raw sewage	10,000x	1,000,000x	10,000,000x

## MPN results

Use the number of positive tubes to find the MPN for each 100 mL from [Table 2](#). [Table 2](#) and [Table 3](#) are for undiluted samples that are 10 mL for each tube. The values are 95 percent confidence limits.

**Example:** Six of the 10 tubes showed a positive response. The MPN for each 100 mL is 9.2.

**Note:** If a test is not used for USEPA reporting, use five broth tubes instead of 10. Refer to [Table 3](#).

**Table 2 MPN table for 10 tubes**

Number of positive tubes	MPN for each 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

**Table 2 MPN table for 10 tubes (continued)**

Number of positive tubes	MPN for each 100 mL
9	23.0
10	> 23.0

**Table 3 MPN table for five tubes**

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

### Example calculation

Do the steps that follow to find the MPN index:

1. Find the MPN index from the positive tubes of the three sets of dilutions. Refer to [Table 4](#).
2. Multiply the MPN index by the Lowest Dilution Factor (LDF).

**Example:** A sample was diluted into three different buffered dilution bottles with these dilutions: 10x, 100x and 1000x. Five tubes were filled from each dilutions with 15 tubes total. The first group of tubes with the 10x dilution had four tubes with gas. The second group of tubes with the 100x dilution had two tubes with gas. The third group of tubes with the 1000x dilution had one tube with gas. The MPN index from [Table 4](#) for four, two and one positive tubes = 26. The coliform result for the sample is:  $26 \times 10 = 260$  coliforms for each 100 mL of sample.

**Table 4 MPN index for dilution groups (for each 100 mL)**

Number of positive tubes			MPN index	Number of positive tubes			MPN index
Dilution group 1	Dilution group 2	Dilution group 3		Dilution group 1	Dilution group 2	Dilution group 3	
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



**Table 4 MPN index for dilution groups (for each 100 mL) (continued)**

Number of positive tubes			MPN index	Number of positive tubes			MPN index
Dilution group 1	Dilution group 2	Dilution group 3		Dilution group 1	Dilution group 2	Dilution group 3	
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

### Controls for coliform bacteria tests

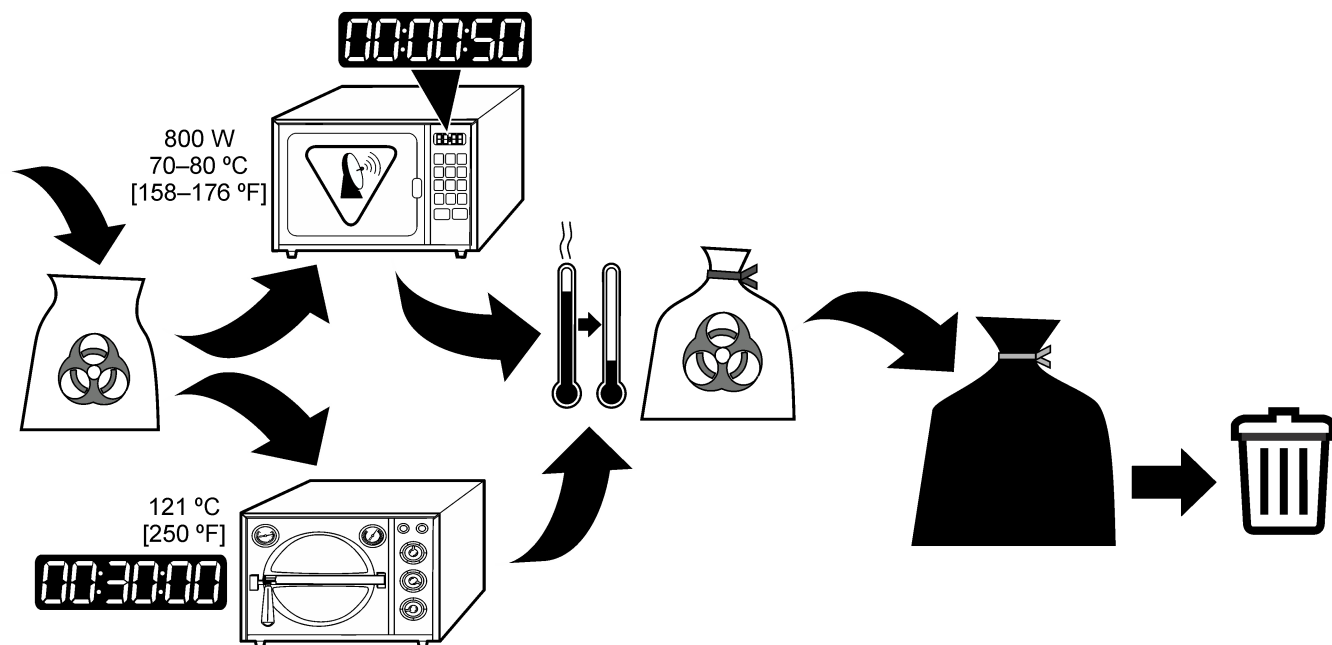
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive 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 Most Probable Number (MPN) method, which is also referred to as the Multiple Tube Fermentation (MTF) technique, uses screw-capped tubes that contain sterile broth medium. The tubes contain an inverted inner vial (a Durham tube) for gas collection. Sample is diluted, added to the tubes and incubated. If coliforms are in the sample, gas is formed in the inner vial. The number of tubes that form gas is used as an estimate of the number of coliform organisms in the sample. Highly turbid samples can be diluted before analysis. It is not necessary to filter the sample.

The Lauryl Tryptose with MUG broth will sense coliforms and *E. coli*. The results are comparable to the traditional MPN fecal coliform tests. The results (of the Lauryl Tryptose with MUG broth method) are received much faster than the traditional MPN fecal coliform tests. No transfer from presumptive to confirmed medium is necessary with the Lauryl Tryptose with MUG broth method. The Lauryl Tryptose with MUG broth medium contains Lauryl Tryptose broth and 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG), a fluorogenic reagent. Tubes positive for *E. coli* will fluoresce when the incubated tubes are examined under a long-wave UV light.

### Consumables and replacement parts

#### Required media and reagents

Description	Quantity/Test	Unit	Item no.
Lauryl Tryptose (with MUG) Broth tubes, concentrated (presumptive and <i>E. coli</i> confirmation)	15	15/pkg	2182115
Dilution water, buffered, 99 mL, sterile <sup>1</sup>	1	25/pkg	1430598

#### Required apparatus

Description	Quantity/Test	Unit	Item no.
Sampling bags, Whirl-Pak <sup>®</sup> with dechlorinating agent, 180 mL, sterilized	1	100/pkg	2075333
UV lamp, long-wave, 115 VAC	1	each	2184300

<sup>1</sup> Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

**Required apparatus (continued)**

Description	Quantity/Test	Unit	Item no.
UV lamp, long-wave, 230 VAC	1	each	2184302
UV blocking eyewear	1	each	SM730-1033
Laboratory incubator, culture, 110 VAC	1	each	2619200
Laboratory incubator, culture, 230 VAC	1	each	2619202
Pipet, serological, 10–11 mL, sterile, disposable	1	25/pkg	209798
Pipet, safety bulb	1	each	1465100
Rack, coliform tube	1	each	221500

**Optional reagents and apparatus**

Description	Unit	Item no.
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Autoclave, 120 VAC	each	2898600
Biohazard bag	200/pkg	2463300
Sampling bags, Whirl-Pak <sup>®</sup> without dechlorinating agent, 720 mL	10/pkg	1437297
Sampling bags, Whirl-Pak <sup>®</sup> 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 with dechlorinating agent	50/pkg	2599150
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
<i>E. coli</i> fluorescence standard	each	2361100
Portable incubator with 12 VDC power socket	each	2569900
Laboratory marker	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 <sup>®</sup> , 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Pipet Aid, 110 VAC recharger, four replacement filters (UL, CSA approved)	each	2551701
Powder Pillows for buffered dilution water (25 of each)	50/pkg	2143166
Sterilization Indicator, Sterikon <sup>®</sup>	15/pkg	2811115
Sterilization Indicator, Sterikon <sup>®</sup>	100/pkg	2811199
Wicks, replacement, for alcohol burner (2087742)	10/pkg	2097810



**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.

On the Worldwide Web – [www.hach.com](http://www.hach.com); E-mail – [techhelp@hach.com](mailto:techhelp@hach.com)

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