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MEL MPN Total Coliform Laboratory

Procedures Manual

09/2018, Edition 1

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Section 1 General information

In no event will the manufacturer be liable for direct, indirect, special, incidental or consequential damages resulting from any defect or omission in this manual. The manufacturer reserves the right to make changes in this manual and the products it describes at any time, without notice or obligation. Revised editions are found on the manufacturer's website.

1.1 Safety information

NOTICE

The manufacturer is not responsible for any damages due to misapplication or misuse of this product including, without limitation, direct, incidental and consequential damages, and disclaims such damages to the full extent permitted under applicable law. The user is solely responsible to identify critical application risks and install appropriate mechanisms to protect processes during a possible equipment malfunction.

Please read this entire manual before unpacking, setting up or operating this equipment. Pay attention to all danger and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment.

Make sure that the protection provided by this equipment is not impaired. Do not use or install this equipment in any manner other than that specified in this manual.

1.2 Use of hazard information

⚠ DANGER

Indicates a potentially or imminently hazardous situation which, if not avoided, will result in death or serious injury.

⚠ WARNING

Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

⚠ CAUTION

Indicates a potentially hazardous situation that may result in minor or moderate injury.

NOTICE

Indicates a situation which, if not avoided, may cause damage to the instrument. Information that requires special emphasis.

1.3 Product overview

The MEL MPN (Most Probable Number) Laboratory is a portable test kit for coliform and *E. coli* bacteria in water samples. The MPN method of analysis uses tubes that contain a broth for bacterial growth. The user adds sample to each tube and incubates the tubes for a specified time. The number of positive tubes determines the most probable number of organisms in the sample. Several MPN tubes contain an inner vial (Durham tube) to collect gas produced by the coliform bacteria. The MPN method is used for very turbid wastewater applications because analysts can dilute rather than filter the sample.

The tubes of broth are not included in the MEL MPN Laboratory. Refer to the consumables and replacement items section of the test procedures to find the media for the applicable test. Make sure to get confirmation media for positive samples and dilution water for wastewater samples.

Refer to the Portable Incubator user manual for operation of the Portable Incubator. Refer to Product Components and the Packaging Guide to assemble the components in the carrying case.

1.4 Product components

Make sure that all components have been received. Refer to the list that follows. If any items are missing or damaged, contact the manufacturer or a sales representative immediately.

- Portable incubator with 12 VDC power socket
- Incubator rack for MPN tubes
- UV lamp, long-wave, portable, 4 watt, battery-operated
- Battery, AA alkaline (4x)
- Thermometer, pocket, –10 to 110 °C (14 to 230 °F)
- Carrying case
- Pipet, serological, 10 mL, sterile, disposable, individually wrapped (50x)
- Pipet safety bulb
- Inoculating loops, sterile, disposable, 10 µL (50x)
- Sampling bags, Whirl-Pak with dechlorinating agent, 180 mL (50x)
- Laboratory marker

Section 2 Bacteria analysis

The amount and type of bacteria in water samples is routinely measured to find whether the water contains disease-causing organisms. All tests for bacteria use a nutritional broth or agar and incubation at a specific temperature to grow the target organism. Sterile equipment and careful handling techniques are necessary to prevent contamination of the sample.

2.1 About indicator organisms

Bacterial pathogens that cause serious diseases are difficult to detect in water supplies and include long and complex test procedures. Thus, tests for indicator organisms that have a fecal origin such as coliform bacteria are commonly used. Indicator organisms may not be pathogenic but are present when pathogens are present and absent when pathogens are absent.

Total coliform bacteria are commonly used as indicator organisms in potable water supplies in temperate climates. Fecal coliform bacteria, and more specifically Escherichia coli, are commonly used as indicator organisms for non-potable water, wastewater, bathing water and swimming water.

In tropical climates, indigenous *Escherichia coli (E. coli)* bacteria give positive results in total coliform tests even in clean water sources where no fecal contamination exists. Thus, other bacteria that are associated with fecal contamination, such as hydrogen sulfide-producing bacteria, are used as an indicator organism.

2.2 Presumptive and confirmation procedures

Most test methods for bacteria begin with a presumptive test procedure. If the result from the presumptive test is positive, a confirmation procedure must be completed. Some media is selective for the target organism and a confirmation test is not required. For example, the m-ColiBlue24® broth and broth that contains MUG is selective for *E. coli*.

- Presumptive test—a positive result is an indication of the target organism but can include a false positive result.
- Confirmation test—the cultured bacteria from the presumptive test are used to inoculate the confirmation media. The confirmation media is more selective for the target organism and may use a higher incubation temperature.

2.3 Preparation for bacteria tests

Good laboratory technique is necessary for bacteria tests. To make sure that the results are reliable, collect and preserve samples carefully. Use high-quality laboratory equipment and ready-to-use media to save time and prevent errors.

2.3.1 Prepare the work area

- Wash hands thoroughly with soap and water.
- Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. A small propane torch can be used to flame-sterilize metal faucets that are used for sample collection.
- Set the incubator to the temperature that is specified by the test procedure. Typically 35 ± 0.5 °C (95 ± 0.9 °F) is used for total coliforms and enterococci and 44.5 ± 0.2 °C (112.1 ± 0.4 °F) is used for fecal coliforms.

2.3.2 Sterilize laboratory equipment

All materials that are used to contain or transfer samples must be sterile to prevent contamination and false results. Use pre-sterilized, disposable laboratory equipment and media to save time and minimize errors. When numerous analyses must be completed on a routine basis, sterilization of non-disposable materials with an autoclave is recommended.

1. Wash sample containers and any necessary equipment with hot water and detergent. Some procedures use equipment such as pipets, petri dishes, a filter holder with stopper and a graduated cylinder.
2. Rinse containers and equipment several times with tap water and then with deionized water.
3. If the water to be sampled has been disinfected by some entity before collection, add the contents of one Dechlorinating Reagent Powder Pillow for each 125 mL of container volume (for 250-mL sample containers, use two powder pillows.)
4. Prepare all equipment for the autoclave as follows:
   a. Loosely install the caps on sample bottles and put foil or paper on caps and bottle necks.
   b. Put foil or paper over the openings of graduated cylinders.
   c. Insert the filter funnel base into an autoclavable rubber stopper that will fit the filter flask.
   d. Put heavy wrapping paper around the two parts of the filter funnel assembly and seal with masking tape.
   e. Put paper around petri dishes (borosilicate glass) or put in aluminum or stainless steel cans.
5. Put the containers and equipment in the autoclave. Steam sterilize the containers and equipment at 121 °C (250 °F) for 15 minutes. Glass sample containers can be sterilized with hot air at 170 °C (338° F) for 1 hour.
6. When sterilization is complete, put on sterile gloves and tighten the caps on the containers and equipment. Put the labware in a clean environment until needed.

2.4 Sample collection and preservation

Collect a sufficient volume of sample for analysis (usually a minimum of 100 mL of sample). The World Health Organization guidelines recommend 200 mL per sample. Standard Methods for the Examination of Water and Wastewater recommends 100 mL per sample.

No dechlorination is necessary if the sample is added directly to the growth medium on site. If not, add a dechlorinating reagent to the samples to remove the chlorine residual. Sodium thiosulfate that has been sterilized in the collection vessel is used to remove chlorine residual. Transport the sample for analysis immediately after collection.

Analyze the samples as soon as possible after collection. If the analysis cannot be started immediately, keep the sample at or below 10 °C (50 °F), but do not freeze the sample. The maximum time between sample collection and incubation is 8 hours. Failure to collect and transport samples as specified will cause inaccurate results. Refer to the local regulatory agency for the most current holding times and temperatures.

Use sterilized plastic bags or disposable bottles

Use pre-sterilized Whirl-Pak® bags or bottles for sample collection. If the sample has been disinfected, use bags or bottles that contain a dechlorinating agent. Bags or bottles that contain dechlorinating reagent can be used for all samples because the dechlorinating reagent does not interfere with untreated samples. As an alternative, use autoclavable glass or plastic bottles.

Write the sample number, dilution, date and other necessary information on each sample container. Use aseptic technique to prevent internal contamination of the sample container.

Faucets, spigots, hydrants or pumps

1. Let the water flow at a moderate rate for 2 to 3 minutes (potable water).
2. Adjust the flow before the sample collection to prevent spills and splashes. Do not adjust the flow during the sample collection. Do not use valves, spigots and faucets that swivel or leak. Remove any aerators or screens.
3. Collect a minimum of 100 mL of the sample in a sterilized container. Do not fill the sample containers completely. Keep a minimum of 2.5 cm (1 inch) of air space to help mix the sample before analysis.
   Note: *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.*
4. Write the sample information on the container and start the analysis as soon as possible.

Rivers, lakes and reservoirs

1. Do not collect samples near the edge of the river, lake or reservoir.
2. If possible, remove the cap under water. As an alternative, remove the cap, grasp the sample container near the bottom and plunge the container, mouth down, into the water to exclude any surface scum.
3. Fill the container entirely under water. Put the mouth of the container into the current or, in non-flowing water, tilt the container slightly and let the container fill slowly. Do not rinse the container before use.
4. Write the sample information on the container and start the analysis as soon as possible.

2.5 MPN dilution guidelines

It is necessary to dilute non-potable water samples so that an MPN test has a minimum of one positive or one negative tube out of the 15 tubes.

If all of the tubes in an MPN test are positive, dilute the samples and do the test again until the dilution series gives both positive and negative tubes. Refer to the dilution tables in Table 1 and Serial sample dilutions on page 8.

If all of the tubes in an MPN test are positive or negative, the sample was not correctly diluted. Make larger dilutions if all MPN tubes are positive. Make smaller dilutions if all MPN tubes are negative. Do the test again with the correct dilutions.

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

2.5.1 Sterile buffered dilution water

Use dilution water that is buffered to a neutral pH and sterilized for microbiological testing. Hach dilution water is recommended for dilution of most non-potable and wastewater samples. Each bottle contains 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 dilution). Before and after the sample is added, make sure to fully mix the bottles. The dilution factor of an undiluted sample = 1.

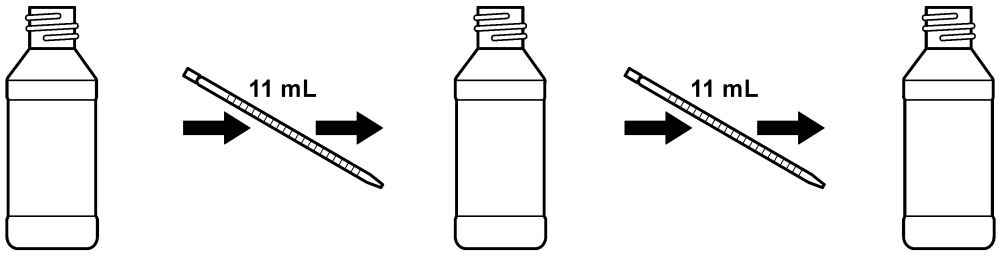
2.5.2 Serial sample dilutions

Do the steps that follow to make serial dilutions of the sample. Refer to Table 2 thru Table 7 to find the number of dilutions for different sample types.

Example: For final wastewater effluent, chlorinated, 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 10x 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 shown in the tables that follow.
   Note: *Do not shake the sample too vigorously because this will injure or stress the organisms.*

Table 2 Swimming pool water, chlorinated—Lowest Dilution Factor (LDF) = 1



Bottle A	Bottle B	Bottle C
Undiluted sample	10x dilution	100x dilution
Inoculate 5 tubes	99 mL dilution water	99 mL dilution water
	Inoculate 5 tubes	Inoculate 5 tubes

Table 3 Bathing beach water; lake water, unpolluted river water—LDF = 10

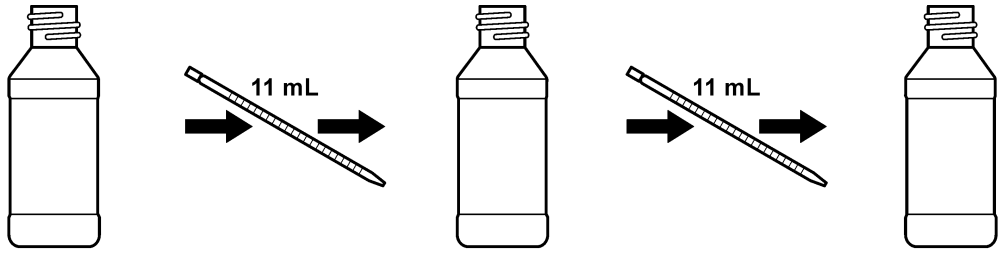
		
Bottle B (from Table 2) 10x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle C** 100x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle D** 1000x dilution 99 mL dilution water Inoculate 5 tubes

Table 4 Final wastewater effluent, chlorinated—LDF= 100

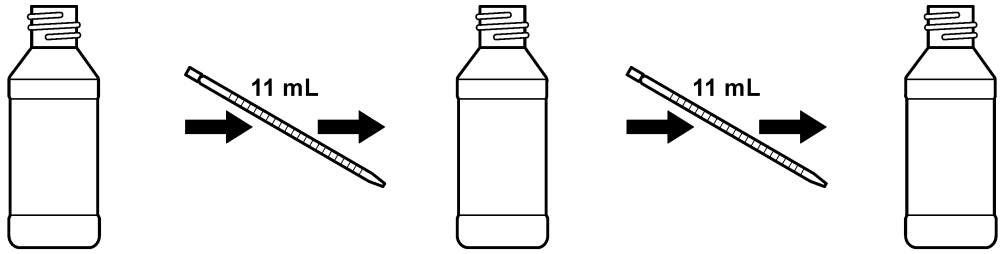
		
Bottle C (from Table 3) 100x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle D** 1000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle E** 10,000x dilution 99 mL dilution water Inoculate 5 tubes

Table 5 River water, polluted—LDF = 1000

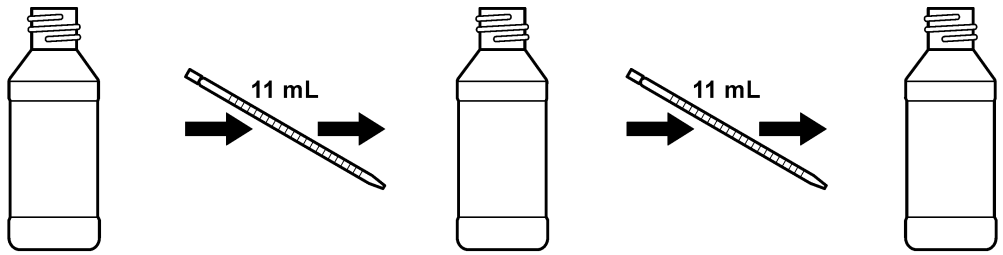
		
Bottle D (from Table 4) 1000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle E** 10,000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle F** 100,000x dilution 99 mL dilution water Inoculate 5 tubes

Table 6 Storm water or unchlorinated final wastewater effluent—LDF= 10,000

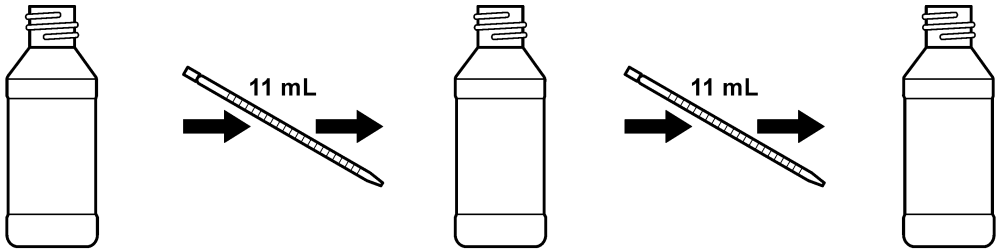
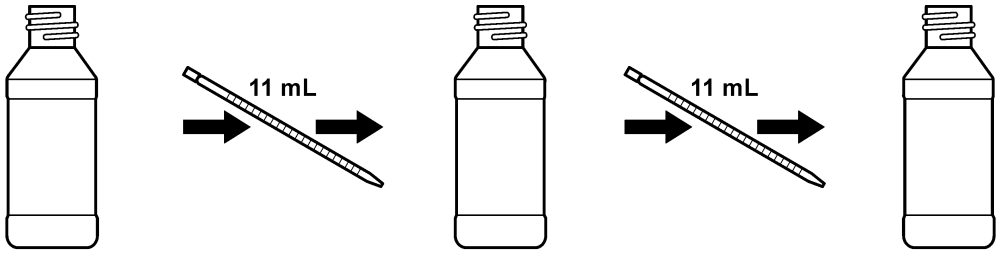
		
Bottle E (from Table 5) 10,000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle F** 100,000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle G** 1,000,000x dilution 99 mL dilution water Inoculate 5 tubes

Table 7 Raw sewage—LDF= 100,000

		
Bottle F (from Table 6) 100,000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle G** 1,000,000x dilution 99 mL dilution water Inoculate 5 tubes	**Bottle H** 10,000,000x dilution 99 mL dilution water Inoculate 5 tubes

2.6 Controls for coliform bacteria tests

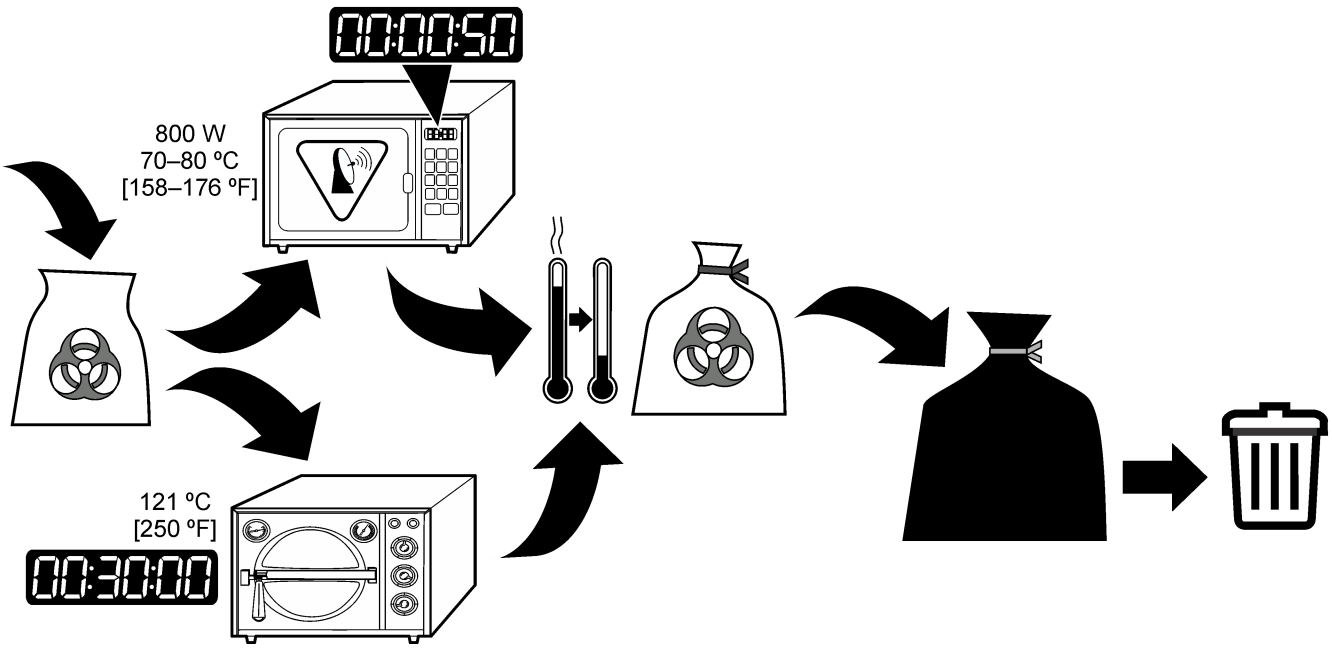
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive control.

2.7 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



Microbiological Procedures

Coliforms, Total, Fecal and *E. Coli*

USEPA[1] Lauryl Tryptose Broth presumptive test **Method 8001**

BGB, EC Medium and EC/MUG confirmation **Most Probable Number (MPN)**

Potable water

Scope and application: For potable water.

[1] 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).

Test preparation

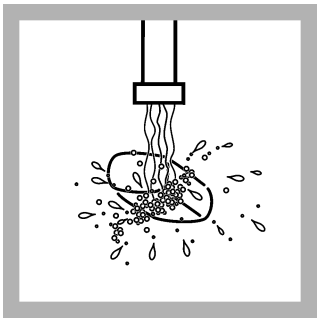
Before starting

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 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Read the section on Sample collection and preservation on page 6.
For the presumptive test, use Lauryl Tryptose broth. For the total coliform confirmation test, use Brilliant Green Bile (BGB) broth. For the fecal coliform confirmation test, use EC Medium broth. For the *E. coli* confirmation test, use EC Medium with MUG broth.
Potable water should not contain coliform bacteria. Do not dilute samples. Use the 10-tube MPN test.
For USEPA reporting, it is necessary to inoculate the confirmation tubes with an inoculation loop. Cap transfer is not permitted.
If the test is not used for USEPA reporting, use five broth tubes instead of 10 broth tubes. Refer to MPN results on page 21 to find the results of the five-tube test. The five-tube test cannot be used for USEPA reporting.
If all 10 tubes (for the 10-tube MPN test) of the confirmed coliform test are negative, the sample is accepted as meeting bacterial standards. To make sure that the sample results are interpreted in accordance with appropriate standards and regulations, contact the local, county, state or federal regulatory agency.
Refer to Bacteria disposal on page 10 for instructions on correct bacteria disposal.

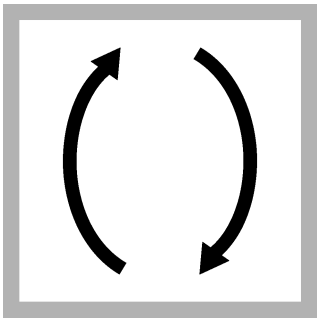
Items to collect

Description	Quantity
Lauryl Tryptose broth tubes	10
Brilliant Green Bile (BGB) broth tubes (total coliform confirmation)	varies
EC Medium broth tubes (fecal coliform confirmation)	varies
EC Medium with MUG broth tubes (fecal coliform and *E. coli* confirmation)	varies
Pipet, serological, 10–11 mL, sterile	1
Pipet filler bulb	1
Inoculating loop	1
Incubator	1
MPN tube incubator rack	1

Presumptive test for coliform bacteria

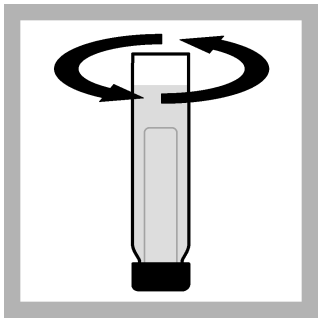


1. Wash hands thoroughly with soap and water.

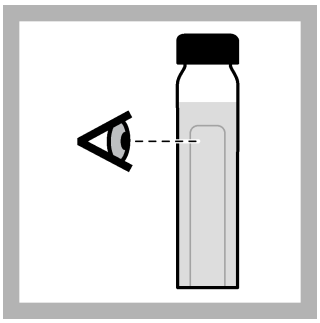


2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.

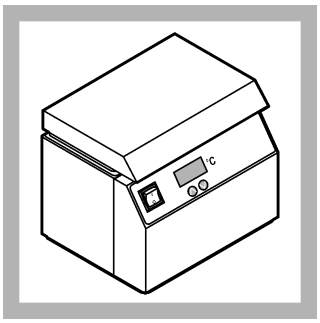
3. Use a sterile pipet to transfer 10 mL of sample into each of the 10 tubes of Lauryl Tryptose broth. Do not touch the open end of the tubes or the inner surface of the caps. Immediately replace and tighten the screw cap on each tube.



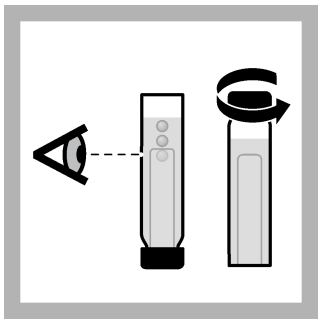
4. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



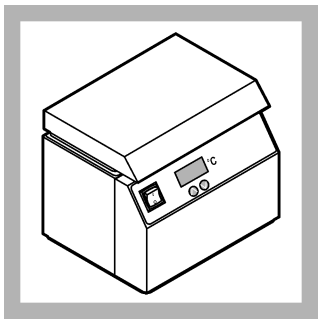
5. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



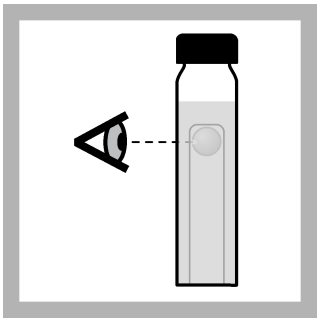
6. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



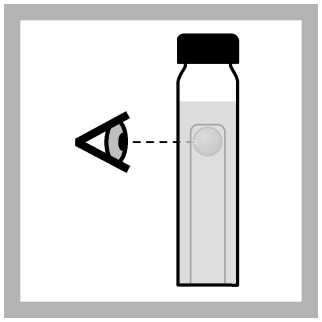
7. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



8. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 23 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



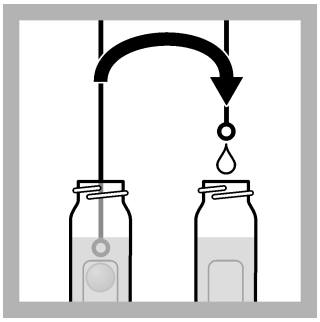
9. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be seen, put the tubes in the incubator for 24 ± 2 hours (48 ± 3 hours total) and examine the tubes again.



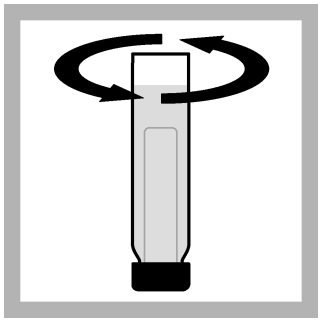
10. Count the number of tubes that contain gas in the inner vial.
Complete a confirmation test for the tubes that contain gas. The confirmation test determines if total coliforms, fecal coliforms or *E. coli* are in the sample. The confirmation test is used to remove false-positive results that can occur with the presumptive test.

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

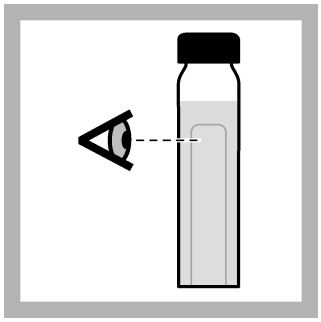
Confirmation test for total coliforms (Brilliant Green Bile broth)



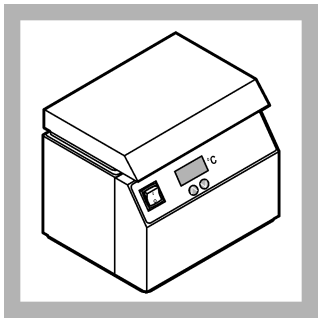
1. From each positive Lauryl Tryptose broth tube, inoculate a Brilliant Green Bile (BGB) broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire.
Put the loop into the positive Lauryl Tryptose broth tube and immediately into a BGB broth tube. Do not touch the rim of the tubes with the loop/wire.
Immediately replace and tighten the screw cap on each tube.



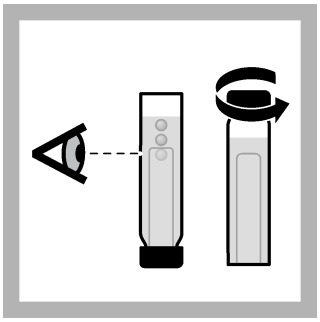
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



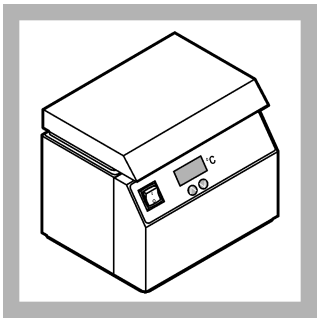
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



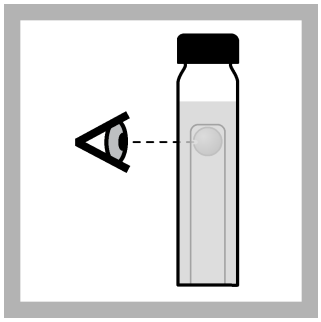
4. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



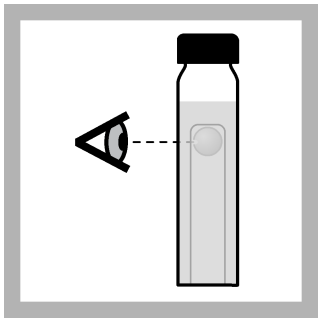
5. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



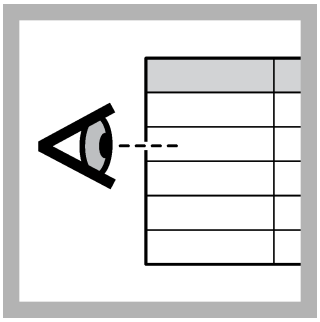
6. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



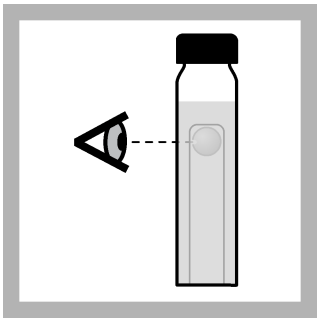
7. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be seen, put the tubes in the incubator for 24 ± 2 hours (48 ± 3 hours total) and examine the tubes again.



8. After 48 ± 3 hours, gently tap each tube and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria.
If none of the tubes contain gas, then the test is negative for total coliform bacteria.

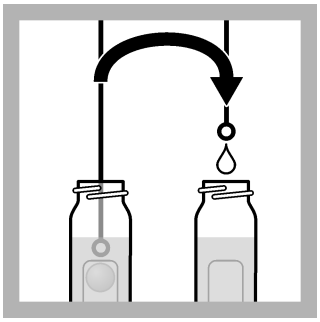


9. Count the number of tubes that contain gas in the inner vial. Refer to Table 1 on page 21 to find the MPN for each 100 mL sample.

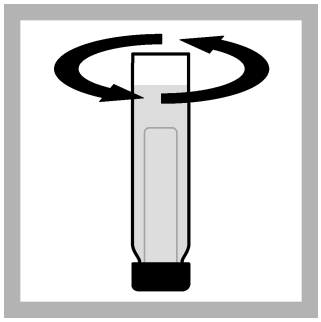


10. If the test is positive for total coliform bacteria, complete a confirmation test for fecal coliform or *E. coli* bacteria (required for USEPA reporting).

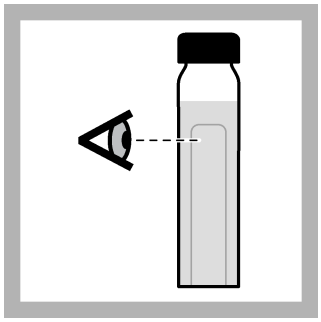
Confirmation test for fecal coliforms (EC Medium)



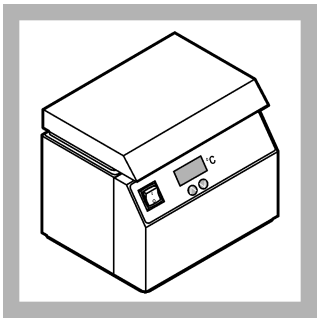
1. From each positive Lauryl Tryptose broth tube, inoculate an EC Medium broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose broth tube and immediately into an EC Medium broth tube. Do not touch the rim of the tubes with the loop/wire. Immediately replace and tighten the screw cap on each tube.



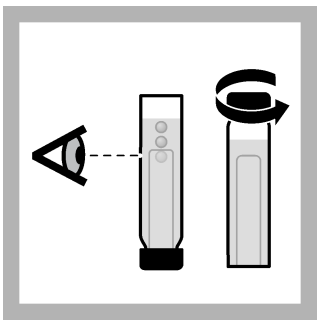
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



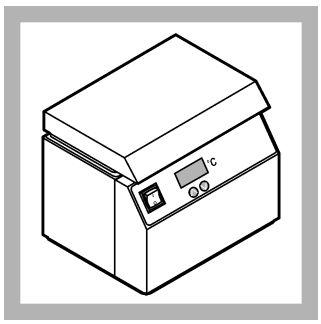
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



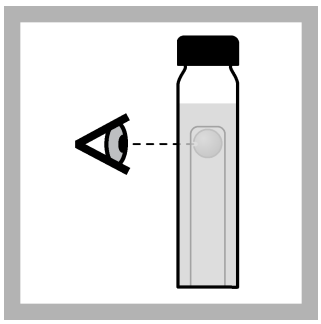
4. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



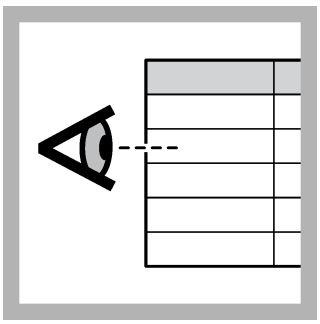
5. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



6. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for an additional 24 ± 2 hours. ***Note:*** *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



7. After 24 ± 2 hours, remove the tubes from the incubator. 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.



8. Count the number of tubes that contain gas in the inner vial. Refer to Table 1 on page 21 to find the MPN for each 100-mL sample.

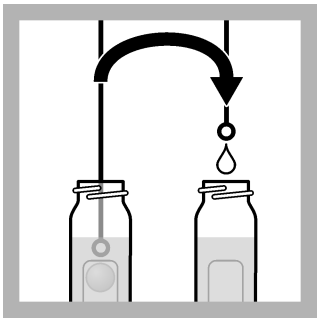
Confirmation test for *E. coli* (EC Medium with MUG)

⚠CAUTION

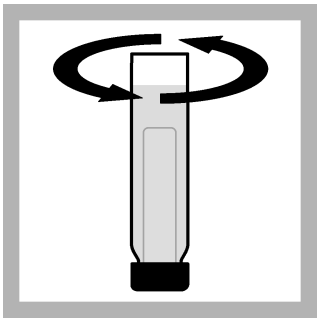
	Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

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.

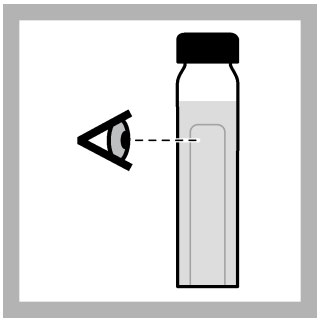
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



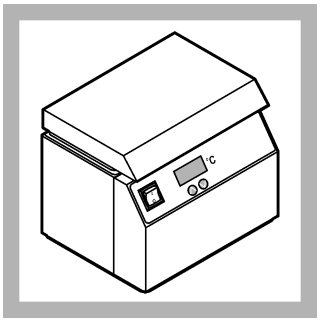
1. From each positive Lauryl Tryptose broth tube, inoculate an EC Medium with MUG broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose tube and immediately into an EC Medium with MUG tube. Do not touch the rim of the tubes with the loop/wire. Immediately replace and tighten the screw cap on each tube.



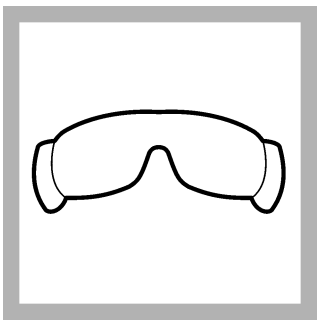
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



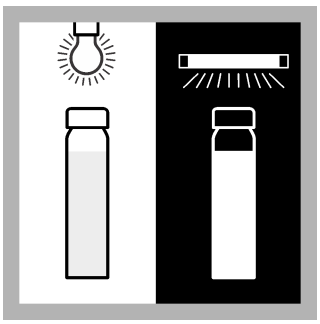
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



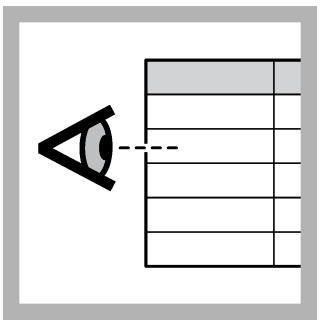
4. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 hours.
Bubbles that form in the inner vials during the first hour are not from bacteria.



5. After 24 ± 2 hours, remove the tubes from the incubator. Put on UV safety goggles



6. Apply UV light to the incubated sample 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* positive confirmation.
If the sample fluoresces, *E. coli* bacteria are in the sample.
If the sample does not fluoresce, there is no *E. coli* in the sample.



7. Count the number of tubes that show fluorescence. Refer to Table 1 on page 21 to find the MPN of the sample (*E. coli* bacteria per 100 mL sample).

MPN results

Use the number of positive tubes to find the MPN for each 100 mL from Table 1. Table 1 and Table 2 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 2.

Table 1 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
9	23.0
10	> 23.0

Table 2 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

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 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. When the EC Medium with MUG broth is used, fluorescence under a long-wave UV lamp shows if *E. coli* is in the sample.

Consumables and replacement items

Required media

Description	Quantity/Test	Unit	Item no.
Lauryl Tryptose Broth MPN tubes, concentrated (presumptive)	10	15/pkg	2101415
Brilliant Green Bile (BGB) broth tubes (total coliform confirmation)	varies	15/pkg	32215

Required media (continued)

Description	Quantity/Test	Unit	Item no.
EC Medium broth tubes (fecal coliform confirmation)	varies	15/pkg	1410415
EC Medium with MUG broth tubes with Durham tubes (fecal coliform and *E. coli* confirmation)	varies	15/pkg	2282415
EC Medium with MUG broth tubes without Durham tubes (*E. coli* confirmation)	varies	15/pkg	2471515

Consumables

Description	Quantity/Test	Unit	Item no.
MEL MPN Consumables Set (50 sterilized inoculating loops, 50 sterilized 10-mL pipets, and 100 sterilized Whirl-Pak Bags)		1	2580200
Inoculating loops, sterile, disposable	varies	25/pkg	2749125
Pipet, serological, 10 mL, sterile, disposable, individually wrapped	1	50/pkg	2092628
Sampling bags, Whirl-Pak with dechlorinating agent, 180 mL, sterilized	1	100/pkg	2075333

Replacement items

Description	Unit	Item no.
Battery, AA, 1.5 V, alkaline	4/pkg	1938004
Case assembly, MEL MPN	each	4780900
Portable incubator with 12 VDC power socket	each	2569900
Laboratory marker	each	2092000
Pipet filler, safety bulb	each	1465100
Portable incubator rack, MPN tubes, holds 39 tubes	each	2580501
Thermometer, pocket, –10 to 110 °C	each	187701
Replacement bulb for portable UV lamp	each	2584600
UV lamp, long-wave, portable, 4 watt	each	2415200

Optional items

Description	Quantity	Item no.
Biohazard bag	200/pkg	2463300
E. coli fluorescence standard	each	2361100
Germicidal cloth	50/pkg	2463200

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FAX: (970) 669-2932

 09/2018

Coliforms, Total, Fecal and *E. Coli*

USEPA[1] Lauryl Tryptose Broth presumptive test **Method 8001A**

BGB, EC Medium and EC/MUG confirmation **Most Probable Number (MPN)**

Non-potable water

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

[1] Most Probable Number Method 8001A for wastewater is USEPA-accepted. Method 8001A 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.

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 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
For the presumptive test, use Lauryl Tryptose broth. For the total coliform confirmation test, use Brilliant Green Bile (BGB) broth. For the fecal coliform confirmation test, use EC Medium broth. For the *E. coli* confirmation test, use EC Medium with MUG broth. The confirmation test is used to eliminate false-positive results that can occur with the presumptive test.
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.
For USEPA reporting, it is necessary to inoculate the confirmation tubes with an inoculation loop. Cap transfer is not permitted.
Read the sections on Sample collection and preservation on page 6 and MPN dilution guidelines on page 7.
Refer to Bacteria disposal on page 10 for instructions on correct bacteria disposal.

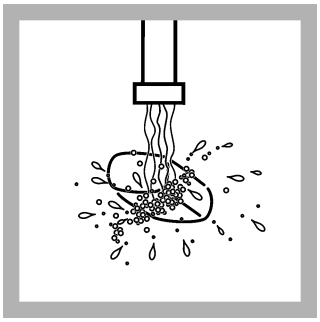
Items to collect

Description	Quantity
Lauryl Tryptose broth tubes	15
Brilliant Green Bile (BGB) broth tubes (total coliform confirmation)	varies
EC Medium broth tubes (fecal coliform confirmation)	varies
EC Medium with MUG broth tubes (fecal coliform and *E. coli* confirmation)	varies
Dilution water, buffered, 99-mL, sterile	3 or more bottles
Pipet, serological, 10–11 mL, sterile	1
Pipet filler bulb	1
Inoculating loop	1

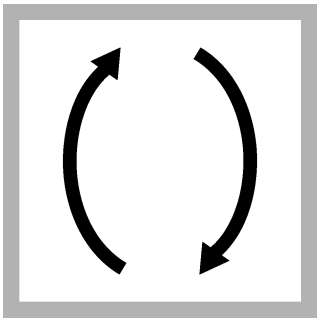
Items to collect (continued)

Description	Quantity
Incubator	1
MPN tube incubator rack	1

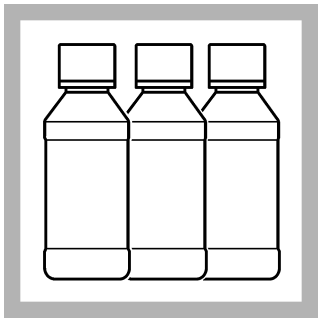
Presumptive test for coliform bacteria (Lauryl Tryptose Broth)



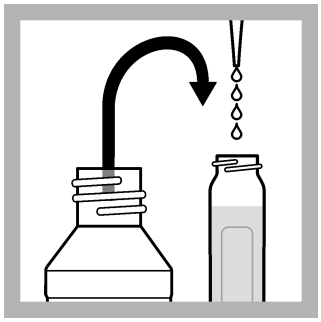
1. Wash hands thoroughly with soap and water.



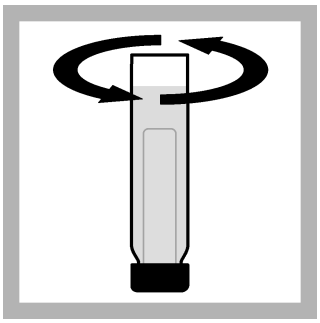
2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.



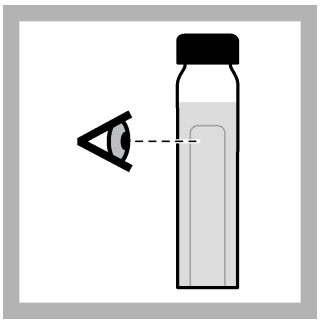
3. Prepare a minimum of three serial dilutions of the sample with sterile buffered dilution water. Refer to MPN dilution guidelines on page 7 for dilution instructions.



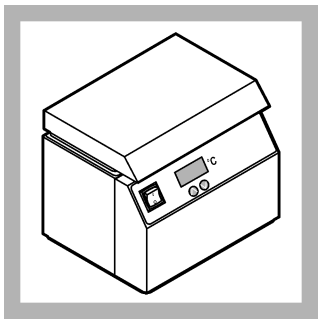
4. Remove the caps from 15 tubes of Lauryl Tryptose broth, one at a time. Use a sterile pipet to add 10-mL portions of each sample dilution into five Lauryl Tryptose broth tubes for the first dilution. Do this two more times for the second and third dilutions. Do not touch the open end of the tubes or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



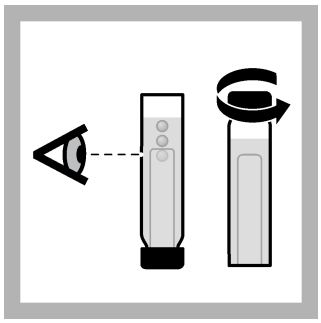
5. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



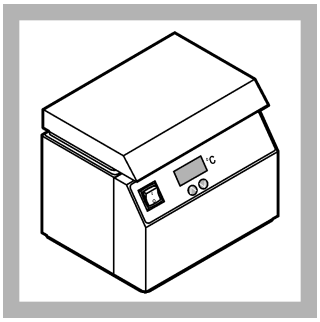
6. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



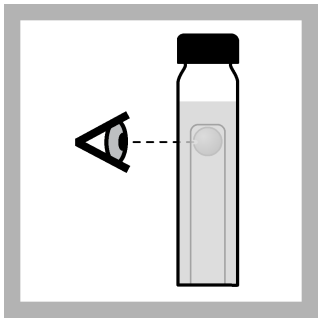
7. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



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.

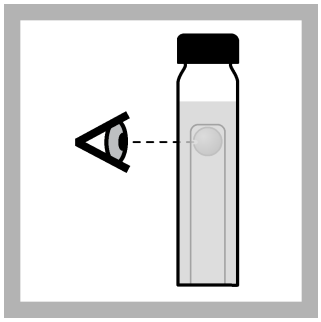


9. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



10. 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 in the sample. Gas in the inner vial is an indication of coliform bacteria.
If no gas can be seen, put the tubes in the incubator for 24 ± 2 hours (for a total of 48 ± 3 hours) and examine the tubes again.

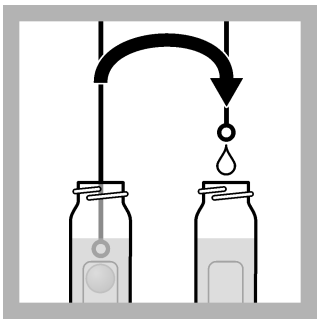
If any gas can be seen, coliform bacteria are in the sample.



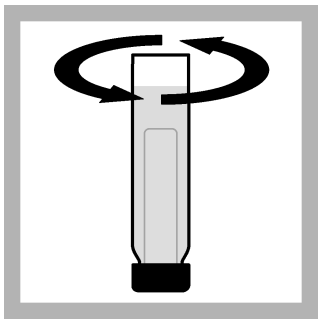
11. Count the number of tubes that contain gas in the inner vial.
Complete a confirmation test for the tubes that contain gas. The confirmation test determines if total coliforms, fecal coliforms or *E. coli* are in the sample. The confirmation test is used to remove false-positive results that can occur with the presumptive test.

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

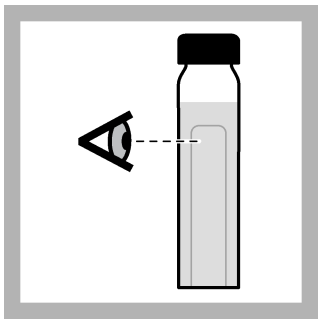
Confirmation test for total coliforms (Brilliant Green Bile Broth)



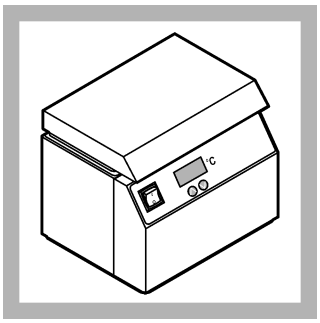
1. From each positive Lauryl Tryptose broth tube, inoculate a Brilliant Green Bile (BGB) broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire.
Put the loop into the positive Lauryl Tryptose broth tube and immediately into a BGB broth tube. Do not touch the rim of the tubes with the loop/wire.
Immediately replace and tighten the screw cap on each tube.



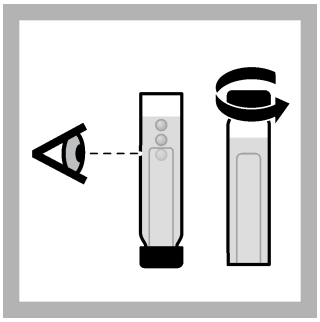
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



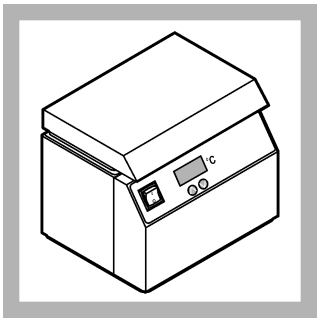
3. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



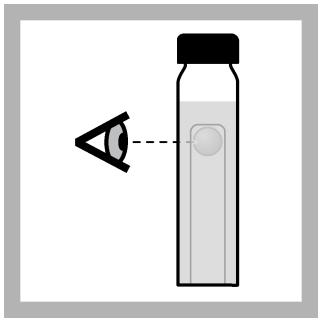
4. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



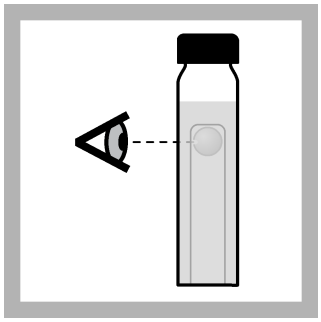
5. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



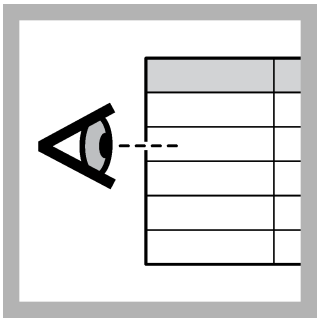
6. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



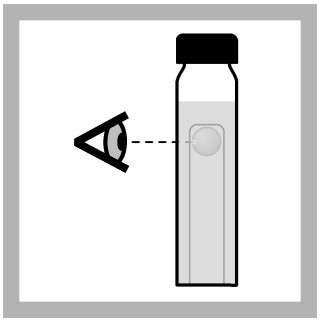
7. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be seen, put the tubes in the incubator for 24 ± 2 hours (48 ± 3 hours total) and examine the tubes again.



8. After 48 ± 3 hours, gently tap each tube and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria.
If none of the tubes contain gas, then the test is negative for total coliform bacteria.

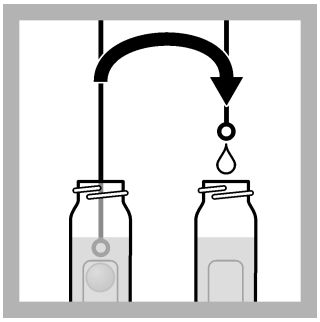


9. Count the number of tubes that contain gas. Refer to Table 1 on page 29 to find the MPN index for each 100 mL sample.

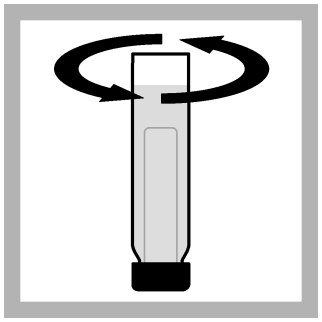


10. If the test is positive for total coliform bacteria, complete a confirmation test for fecal coliform or *E. coli* bacteria (required for USEPA reporting).

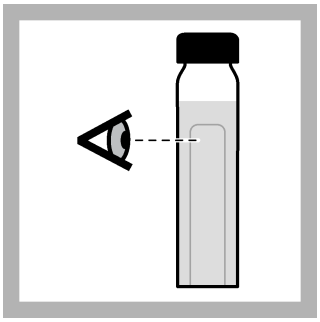
Confirmation test for fecal coliforms (EC Medium)



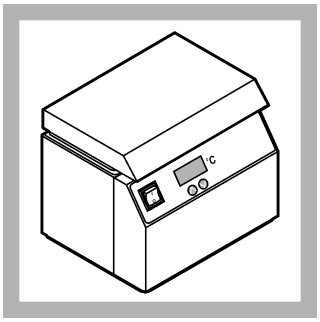
1. From each positive Lauryl Tryptose broth tube, inoculate an EC Medium broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose broth tube and immediately into an EC Medium broth tube. Do not touch the rim of the tubes with the loop/wire. Immediately replace and tighten the screw cap on each tube.



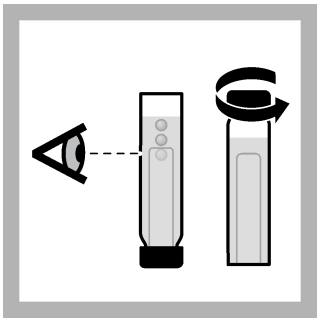
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



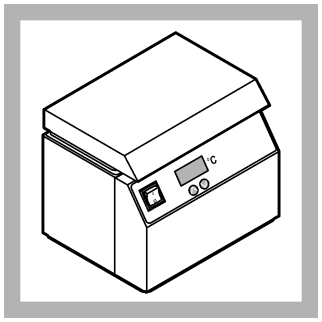
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



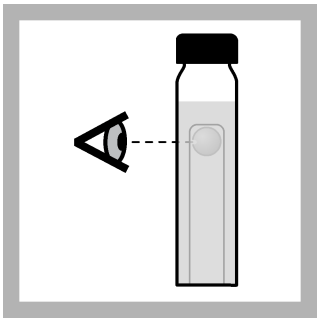
4. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



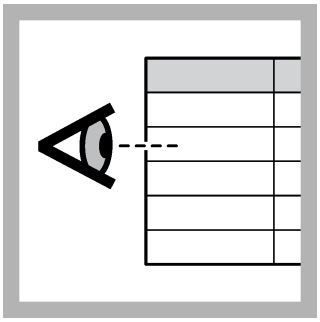
5. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



6. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for an additional 24 ± 2 hours. ***Note:*** *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



7. After 24 ± 2 hours, remove the tubes from the incubator. 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.



8. Count the number of tubes that contain gas in the inner vial. Refer to Table 1 on page 29 to find the MPN for each 100-mL sample.

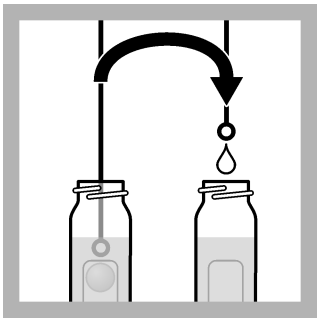
Confirmation test for *E. coli* (EC Medium with MUG broth)

⚠CAUTION

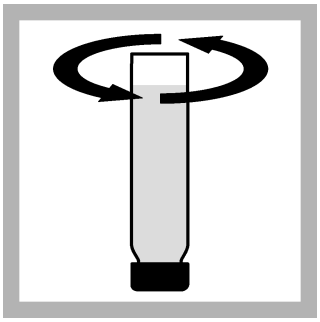
	Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

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.

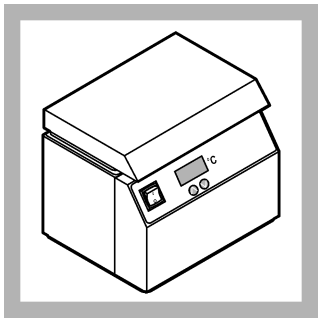
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



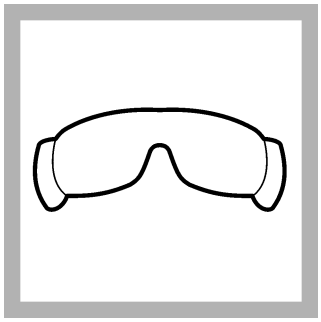
1. From each positive Lauryl Tryptose broth tube, inoculate an EC Medium with MUG broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose broth tube and immediately into an EC Medium with MUG broth tube. Do not touch the rim of the tubes with the loop/wire. Immediately replace and tighten the screw cap on each tube.



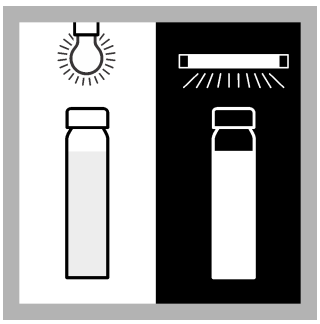
2. Invert the tubes to mix. Gently swirl, if necessary.



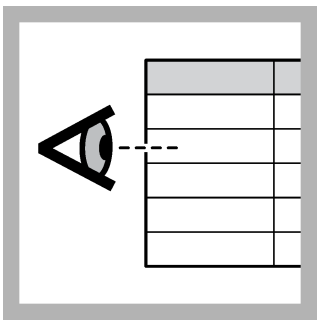
3. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 ± 2 hours.



4. Put on UV safety goggles



5. Apply UV light to the incubated sample that contains MUG broth 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* positive confirmation.
If the sample fluoresces, *E. coli* bacteria are in the sample.
If the sample does not fluoresce, the test is negative for *E. coli*.



6. Count the number of tubes that show fluorescence. Refer to Table 1 on page 29 to find the MPN of the sample.

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 1.
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 1 for four, two and one positive tubes = 26. The coliform result for the sample is: 26 × 10 = 260 coliforms for each 100 mL of sample.

Table 1 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
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

Table 1 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	
4	2	0	22	5	5	4	1600
—	—	—	—	5	5	5	≥1600

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 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. When the EC Medium with MUG broth is used, fluorescence under a long-wave UV lamp shows if *E. coli* is in the sample.

Consumables and replacement items

Required media and reagents

Description	Quantity/Test	Unit	Item no.
Lauryl Tryptose Broth MPN tubes, concentrated (presumptive)	15	15/pkg	2101415
Brilliant Green Bile (BGB) broth tubes (total coliform confirmation)	varies	15/pkg	32215
EC Medium broth tubes (fecal coliform confirmation)	varies	15/pkg	1410415
EC Medium with MUG broth tubes with Durham tubes (fecal coliform and *E. coli* confirmation)	varies	15/pkg	2282415
EC Medium with MUG broth tubes without Durham tubes (*E. coli* confirmation)	varies	15/pkg	2471515
Dilution water, buffered, 99 mL, sterile[1]	1	25/pkg	1430598

Consumables

Description	Quantity/Test	Unit	Item no.
MEL MPN Consumables Set (50 sterilized inoculating loops, 50 sterilized 10-mL pipets, and 100 sterilized Whirl-Pak Bags)		1	2580200
Inoculating loops, sterile, disposable	varies	25/pkg	2749125
Pipet, serological, 10 mL, sterile, disposable, individually wrapped	1	50/pkg	2092628
Sampling bags, Whirl-Pak with dechlorinating agent, 180 mL, sterilized	1	100/pkg	2075333

Replacement items

Description	Unit	Item no.
Battery, AA, 1.5 V, alkaline	4/pkg	1938004
Case assembly, MEL MPN	each	4780900
Portable incubator with 12 VDC power socket	each	2569900
Laboratory marker	each	2092000

[1] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Replacement items (continued)

Description	Unit	Item no.
Pipet filler, safety bulb	each	1465100
Portable incubator rack, MPN tubes, holds 39 tubes	each	2580501
Thermometer, pocket, –10 to 110 °C	each	187701
Replacement bulb for portable UV lamp	each	2584600
UV lamp, long-wave, portable, 4 watt	each	2415200

Optional items

Description	Quantity	Item no.
Biohazard bag	200/pkg	2463300
E. coli fluorescence standard	each	2361100
Germicidal cloth	50/pkg	2463200

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FAX: (970) 669-2932

 09/2018

Coliforms, Total and *E. Coli*

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 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 ± 0.5 °C (95 ± 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 and preservation on page 6 and MPN dilution guidelines on page 7.
Refer to Bacteria disposal on page 10 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

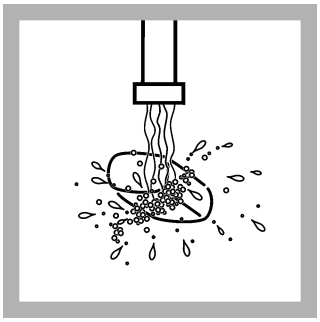
Potable water test for coliforms—total and *E. coli*

⚠ CAUTION

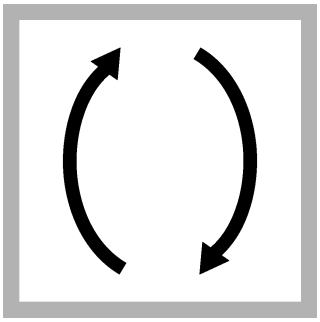
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

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.*

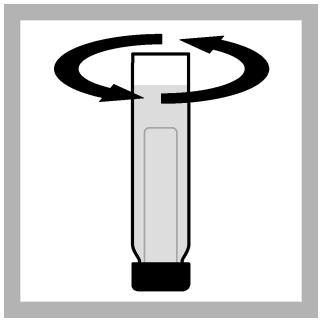


1. Wash hands thoroughly with soap and water.

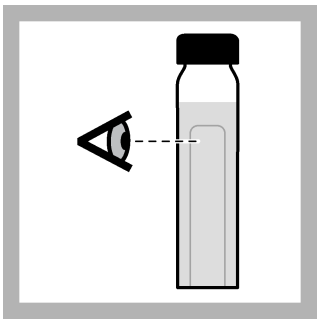


2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.

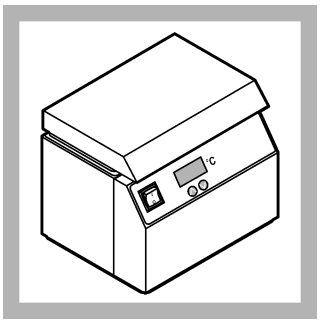
3. Remove the caps from five or 10 tubes of Lauryl Tryptose with MUG broth, one at a time. Use a sterile pipet to transfer 10 mL of sample into each tube. Do not touch the open end of the tubes or the inner surface of the caps. Immediately replace and tighten the screw cap on each tube.



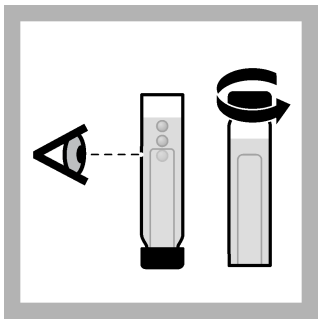
4. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



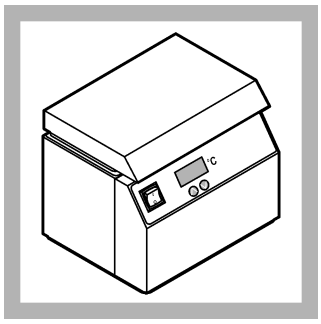
5. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



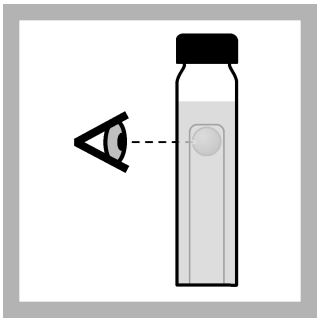
6. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



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.

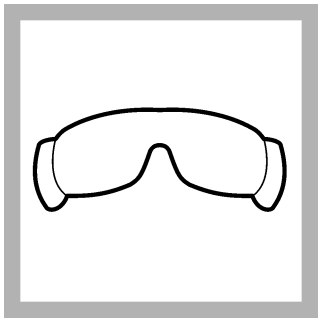


8. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 hours
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*

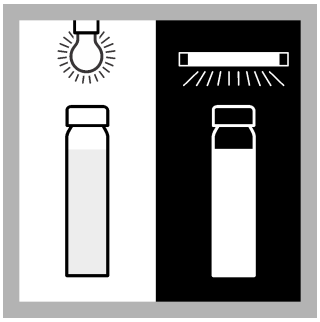


9. After 24 ± 2 hours, remove the tubes from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Gas in the inner vial is an indication of coliform bacteria.
If the tubes are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

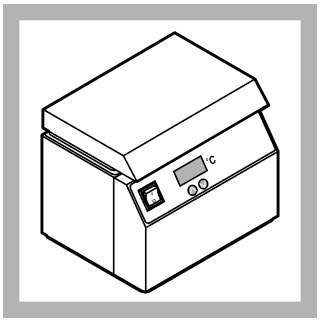
If no gas can be seen, the test is negative for total coliform bacteria.



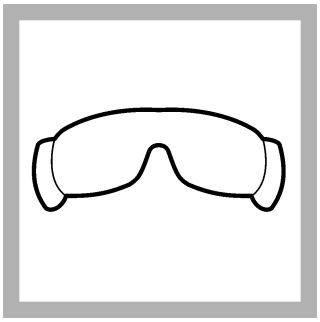
10. Put on UV safety goggles



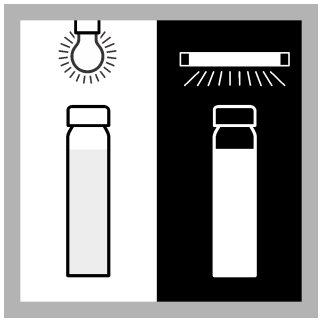
11. Apply UV light to the incubated samples with a long-wave UV lamp. Examine the tubes in a dark area. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* culture for a positive confirmation.
If the sample fluoresces, the test is positive for *E. coli*.
If the sample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



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



13. Put on UV safety goggles



14. After 24 ± 2 hours (for a total of 48 ± 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 37 to find the MPN of the sample.

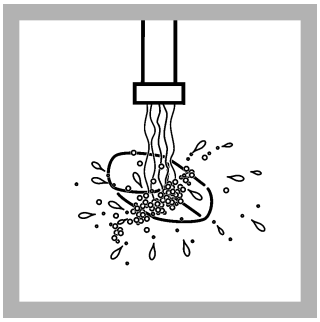
Non-potable water test for coliforms—total and *E. coli*

⚠ CAUTION

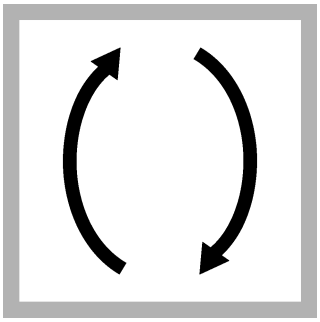
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

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.

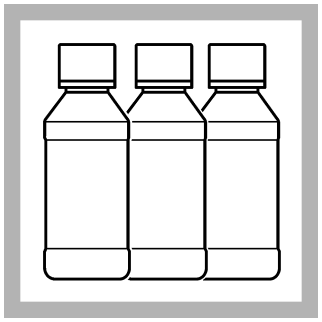
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



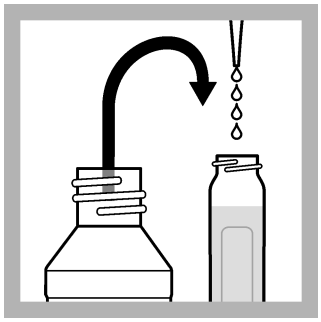
1. Wash hands thoroughly with soap and water.



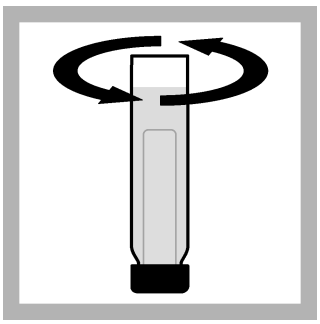
2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.



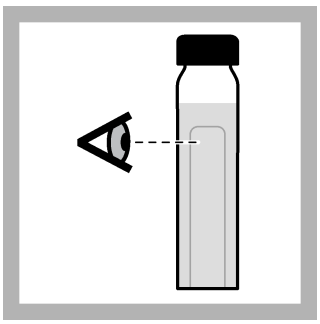
3. Prepare a minimum of three serial dilutions of the sample with sterile buffered dilution water. Refer to MPN dilution guidelines on page 7 for dilution instructions.



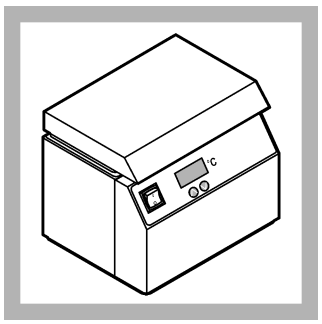
4. Remove the caps from 15 tubes of Lauryl Tryptose with MUG broth, one at a time. Use a sterile pipet to add 10-mL portions of each sample dilution into five tubes for the first dilution. Do this two more times for the second and third dilutions. Do not touch the open end of the tubes or the inner surface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



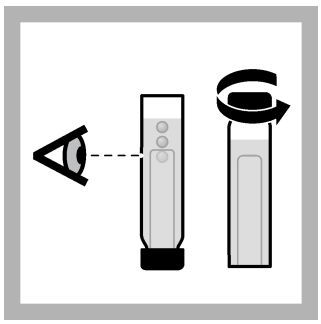
5. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



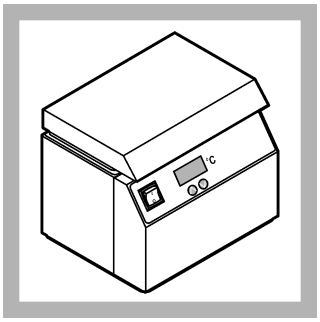
6. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



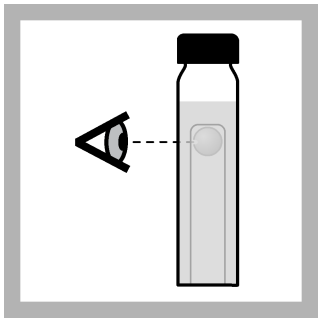
7. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



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.



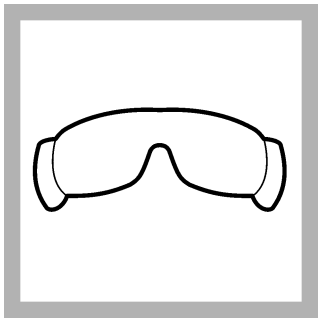
9. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



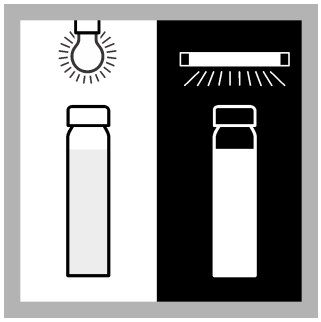
10. After 24 ± 2 hours, tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Gas in the inner vial is an indication of coliform bacteria.

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

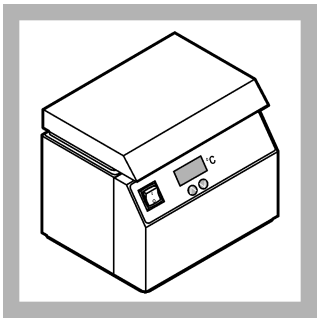
If none of the tubes contain gas, the test is negative for total coliform bacteria.



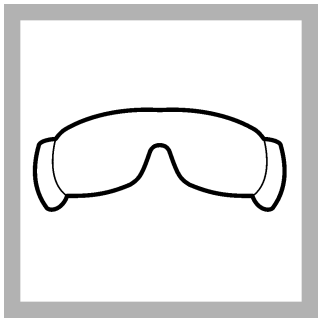
11. Put on UV safety goggles



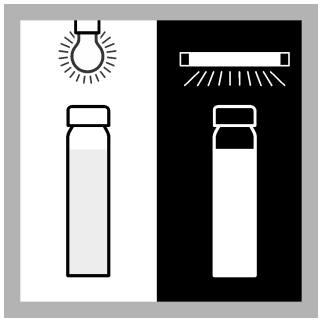
12. Apply UV light to the incubated samples with a long-wave UV lamp. Examine the tubes in a dark area. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* culture for a positive confirmation.
If the sample fluoresces, the test is positive for *E. coli*.
If the sample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



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



14. After 24 ± 2 hours (for a total of 48 ± 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 38 to find the MPN of the sample.

MPN results

Use the number of positive tubes to find the MPN for each 100 mL from Table 1. Table 1 and Table 2 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 2.*

Table 1 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
9	23.0
10	> 23.0

Table 2 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 3.
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 3 for four, two and one positive tubes = 26. The coliform result for the sample is: 26 × 10 = 260 coliforms for each 100 mL of sample.

Table 3 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

Table 3 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	
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

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-ß-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 items

Required media and reagents

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

Consumables

Description	Quantity/Test	Unit	Item no.
MEL MPN Consumables Set (50 sterilized inoculating loops, 50 sterilized 10-mL pipets, and 100 sterilized Whirl-Pak Bags)		1	2580200
Inoculating loops, sterile, disposable	varies	25/pkg	2749125
Pipet, serological, 10 mL, sterile, disposable, individually wrapped	1	50/pkg	2092628
Sampling bags, Whirl-Pak with dechlorinating agent, 180 mL, sterilized	1	100/pkg	2075333

Replacement items

Description	Unit	Item no.
Battery, AA, 1.5 V, alkaline	4/pkg	1938004
Case assembly, MEL MPN	each	4780900
Portable incubator with 12 VDC power socket	each	2569900
Laboratory marker	each	2092000
Pipet filler, safety bulb	each	1465100
Portable incubator rack, MPN tubes, holds 39 tubes	each	2580501
Thermometer, pocket, –10 to 110 °C	each	187701
Replacement bulb for portable UV lamp	each	2584600
UV lamp, long-wave, portable, 4 watt	each	2415200

Optional items

Description	Quantity	Item no.
Biohazard bag	200/pkg	2463300
E. coli fluorescence standard	each	2361100
Germicidal cloth	50/pkg	2463200

[1] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

 09/2018

Coliforms, Fecal

USEPA[1] A-1 Medium

Method 8368

Most Probable Number (MPN)

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

[1] Most Probable Number Method 8368 (A-1 Medium) for non-potable waters is USEPA accepted. Method 8368 meets or exceeds the specification criteria in *Standard Methods for the Examination of Water and Wastewater*, 18th edition, 9221 E. Fecal Coliform Procedure. *USEPA Manual for the Certification of Laboratories Analyzing Drinking Water* states that, "5.5.3. A-1 medium may be used as an alternative to EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. A-1 Medium must not be used for drinking water samples."

Test preparation

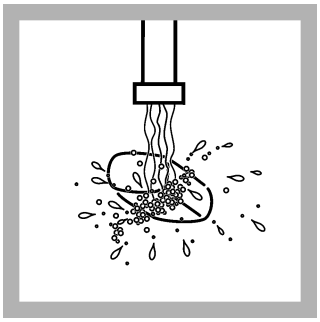
Before starting

Wash hands thoroughly with soap and water.
Make sure that all of the materials that come in contact with samples are sterile.
Set the temperature of the incubator to 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Use a dilute bleach solution, bactericidal spray or dilute iodine solution to clean the work area.
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.
No confirmation is necessary when A-1 Medium broth is used.
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.
Read the section on Sample collection and preservation on page 6.
Refer to MPN dilution guidelines on page 7 to find the number of necessary dilutions based on the sample type.
Refer to Bacteria disposal on page 10 for instructions on correct bacteria disposal.

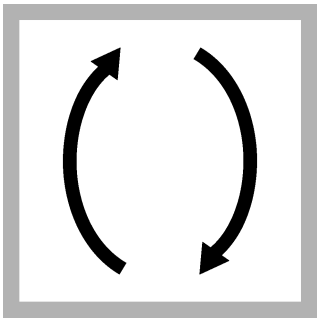
Items to collect

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

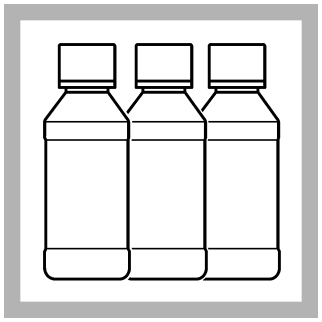
Fecal Coliforms—A-1 Medium Broth



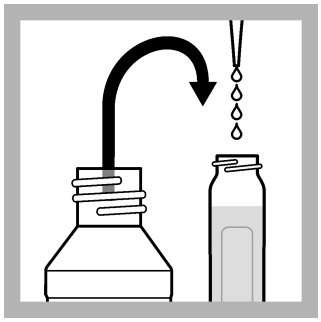
1. Wash hands thoroughly with soap and water.



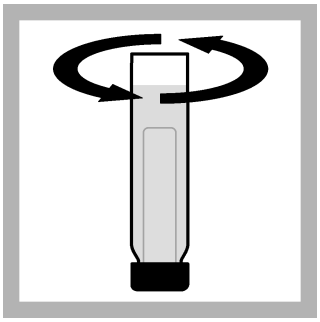
2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.



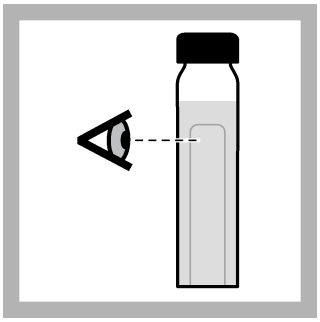
3. Prepare a minimum of three serial dilutions of the sample with sterile buffered dilution water. Refer to MPN dilution guidelines on page 7.



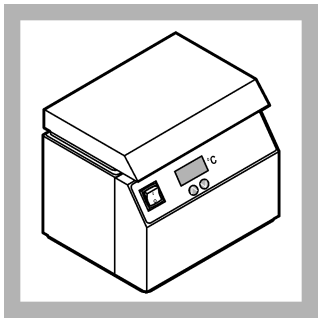
4. Use a sterile pipet to add 10-mL portions of each sample dilution into five A-1 Medium broth tubes for the first dilution. Do this two more times for the second and third dilutions. Do not touch the open end of the tubes or the inner surface of the caps.
Immediately replace and tighten the screw cap on each tube.



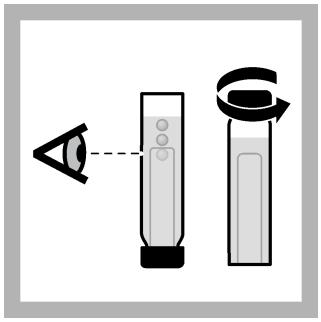
5. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



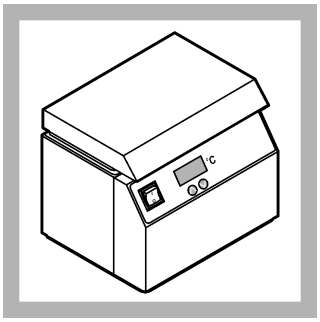
6. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



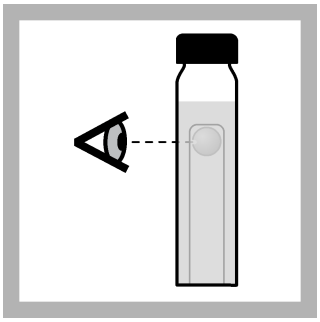
7. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 3 hours.
Bubbles that form in the inner vials during the first hour are not from bacteria.



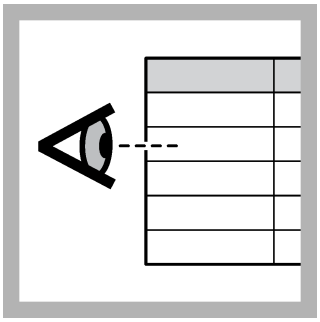
8. After 3 hours, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



9. Incubate the sample at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 21 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



10. After 24 ± 2 hours, remove the tubes from the incubator. 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 no gas is seen, the test is negative for fecal coliform bacteria.



11. Count the number of tubes that contain gas in the inner vial. Refer to Table 1 on page 43 to find the MPN index for each 100 mL.

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 1.
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 1 for four, two and one positive tubes = 26. The coliform result for the sample is: 26 × 10 = 260 coliforms for each 100 mL of sample.

Table 1 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

Table 1 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	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

Summary of method

The Most Probable Number (MPN) method, which is also referred to as the Multiple Tube Fermentation Technique (MTFT), 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. The MPN method is used for the analysis of highly turbid samples by dilution prior to analysis. It is not necessary to filter the sample.

Consumables and replacement items

Required media and reagents

Description	Quantity/Test	Unit	Item no.
A-1 Medium broth tubes	15	15/pkg	2560915
Dilution water, buffered, 99 mL, sterile[1]	1	25/pkg	1430598

Consumables

Description	Quantity/Test	Unit	Item no.
MEL MPN Consumables Set (50 sterilized inoculating loops, 50 sterilized 10-mL pipets, and 100 sterilized Whirl-Pak Bags)		1	2580200
Inoculating loops, sterile, disposable	varies	25/pkg	2749125

[1] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Consumables (continued)

Description	Quantity/Test	Unit	Item no.
Pipet, serological, 10 mL, sterile, disposable, individually wrapped	1	50/pkg	2092628
Sampling bags, Whirl-Pak with dechlorinating agent, 180 mL, sterilized	1	100/pkg	2075333

Replacement items

Description	Unit	Item no.
Battery, AA, 1.5 V, alkaline	4/pkg	1938004
Case assembly, MEL MPN	each	4780900
Portable incubator with 12 VDC power socket	each	2569900
Laboratory marker	each	2092000
Pipet filler, safety bulb	each	1465100
Portable incubator rack, MPN tubes, holds 39 tubes	each	2580501
Thermometer, pocket, –10 to 110 °C	each	187701
Replacement bulb for portable UV lamp	each	2584600
UV lamp, long-wave, portable, 4 watt	each	2415200

Optional items

Description	Quantity	Item no.
Biohazard bag	200/pkg	2463300
E. coli fluorescence standard	each	2361100
Germicidal cloth	50/pkg	2463200

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