Coliforms, Total, Fecal and *E Coli*

DOC316.53.01216

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.
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.
To sterilize an inoculating needle, apply heat to the needle with an alcohol or a Bunsen burner until the needle is red hot. Let the needle cool before use.
If the test is not used for USEPA reporting, use five broth tubes instead of 10 broth tubes. Refer to MPN results on page 7 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 8 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
Alcohol burner	1
Pipet, serological, 10–11 mL, sterile	1
Pipet filler bulb	1
Inoculating loop	1

Items to collect (continued)

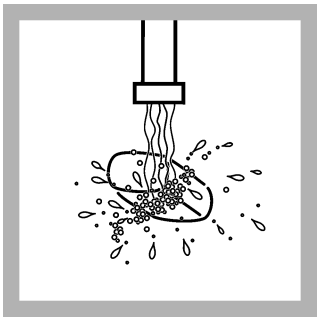
Description	Quantity
Incubator	1
MPN tube incubator rack	1

Refer to Consumables and replacement items on page 9 for order information.

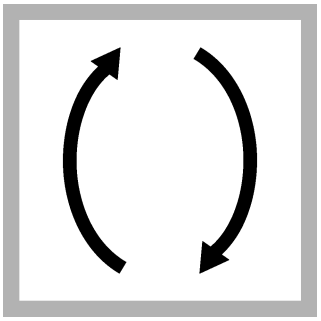
Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak® bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2 to 3 minutes. Remove any screens or aerators. Do not use faucets or spigots that swivel or leak.
- To collect a non-potable sample from a river, lake or reservoir, remove the cap under water. As an alternative, remove the cap and push the container, mouth down, into the water to prevent the collection of surface scum. Fill the container entirely under water. Put the mouth of the container into the current. Put the cap back on the container.
- Collect a minimum of 100 mL of sample and keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If the analysis cannot be started immediately, keep the sample at or below 10 °C (50 °F) for up to 6 hours. Do not let the sample freeze.
- Failure to collect and transport samples correctly will cause inaccurate results.

Presumptive test for coliform bacteria

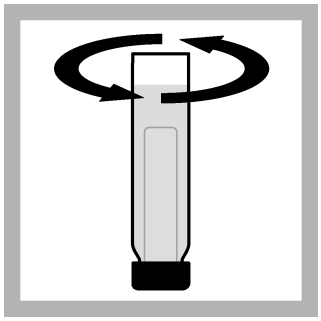


1. Wash hands thoroughly with soap and water.

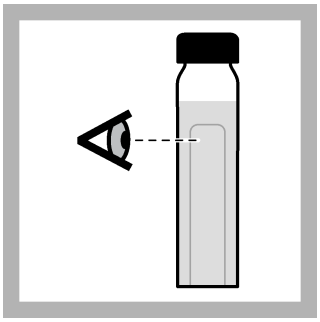


2. Invert the sample for 30 seconds (approximately 25 times) to make sure that the sample is mixed well.

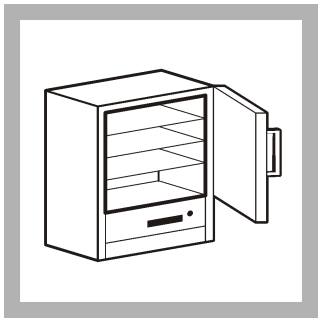
3. Use a sterile pipet to transfer 10 mL of sample into each of the 10 tubes of Lauryl Tryptose broth. Do not touch the open end of the tubes or the inner surface of the caps. Immediately replace and tighten the screw cap on each tube.



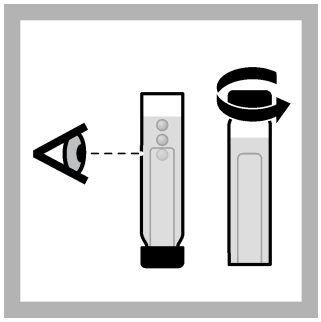
4. Invert the tube. While the tube is inverted, gently swirl until the sample is fully mixed with the nutrient medium.



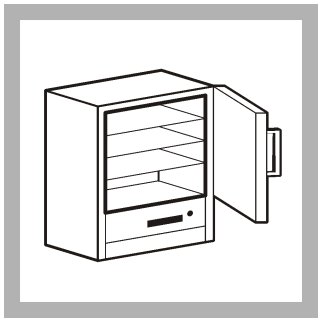
5. Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.



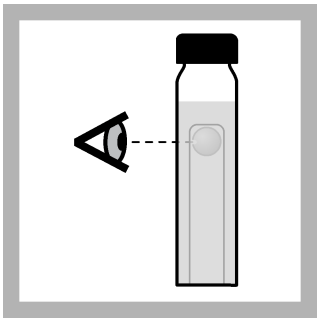
6. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



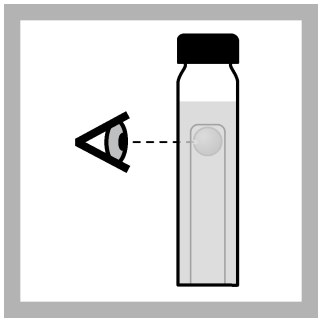
7. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



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.*



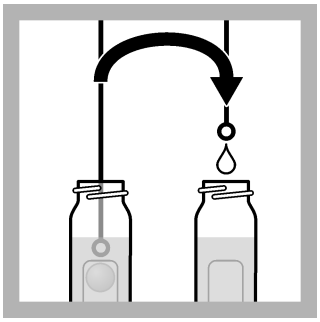
9. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be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



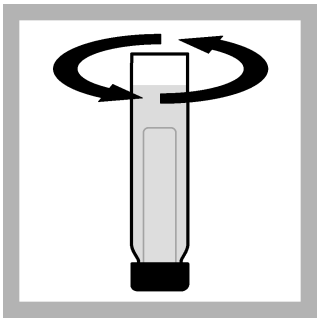
10. Count the number of tubes that contain gas in the inner vial.
Complete a confirmation test for the tubes that contain gas. The confirmation test determines if total coliforms, fecal coliforms or *E. coli* are in the sample. The confirmation test is used to remove false-positive results that can occur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

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

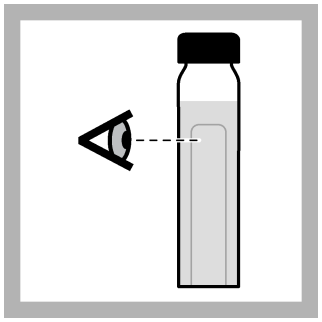
Confirmation test for total coliforms (Brilliant Green Bile broth)



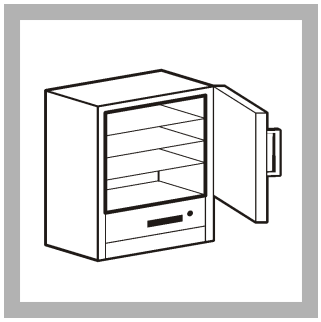
1. From each positive Lauryl Tryptose broth tube, inoculate a Brilliant Green Bile (BGB) broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose broth tube and immediately into a BGB broth tube. Do not touch the rim of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



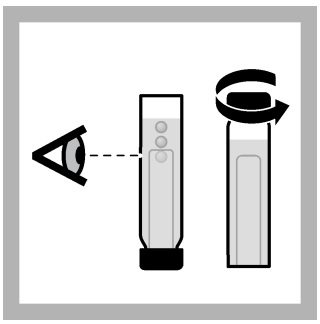
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



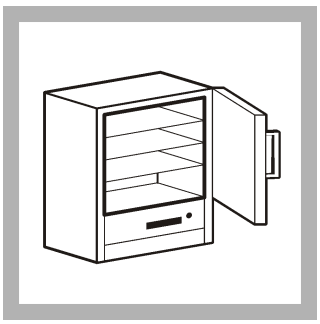
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



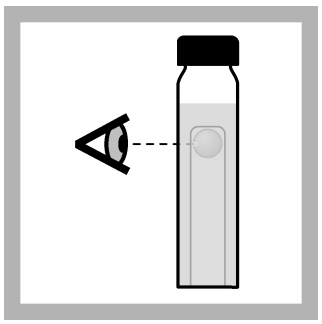
4. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



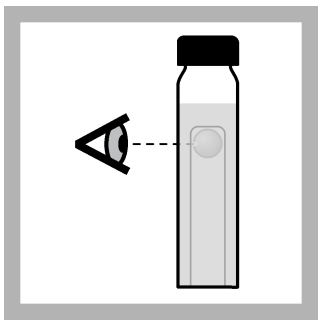
5. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



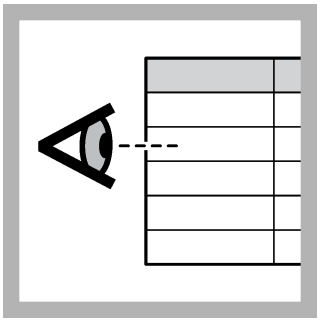
6. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



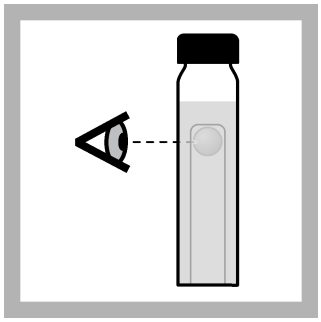
7. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be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

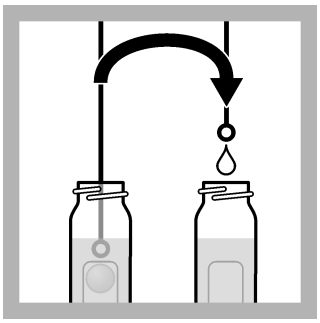


9. Count the number of tubes that contain gas in the inner vial. Refer to Table 1 on page 8 to find the MPN for each 100 mL sample.

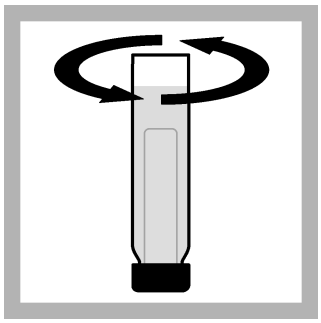


10. If the test is positive for total coliform bacteria, complete a confirmation test for fecal coliform or *E. coli* bacteria (required for USEPA reporting).

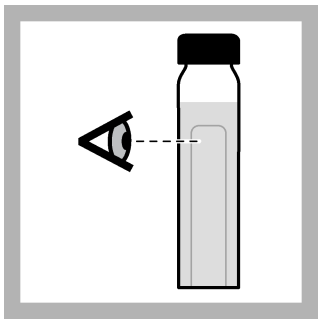
Confirmation test for fecal coliforms (EC Medium)



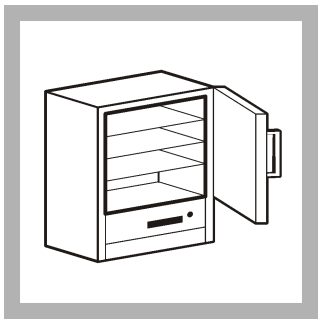
1. From each positive Lauryl Tryptose broth tube, inoculate an EC Medium broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose broth tube and immediately into an EC Medium broth tube. Do not touch the rim of the tubes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



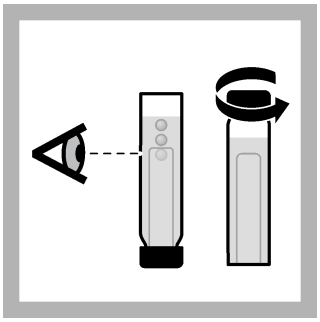
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



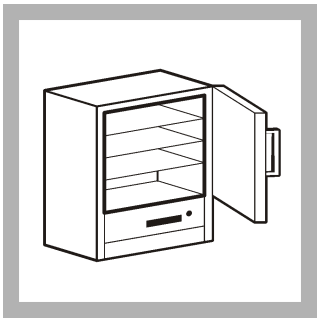
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



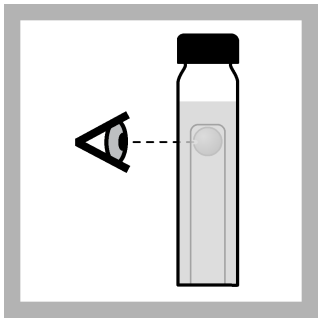
4. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



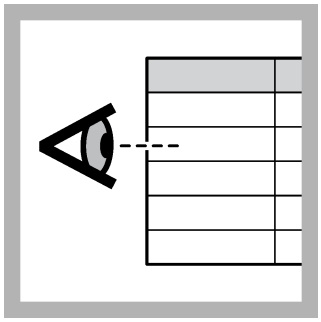
5. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



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 8 to find the MPN for each 100-mL sample.

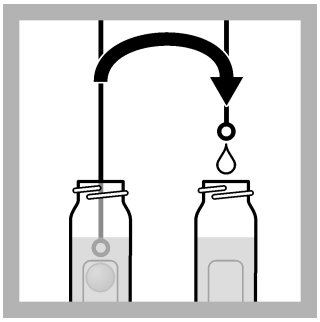
Confirmation test for *E. coli* (EC Medium with MUG)

⚠CAUTION

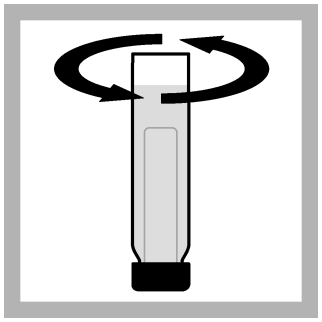
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

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.

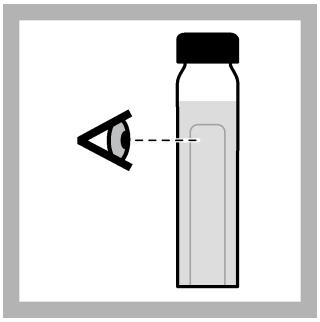
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



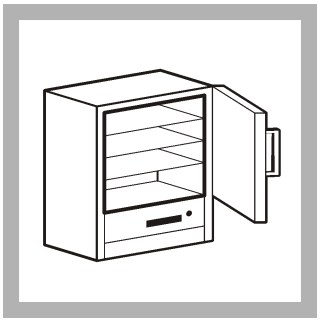
1. From each positive Lauryl Tryptose broth tube, inoculate an EC Medium with MUG broth tube. Use a sterile, disposable inoculation loop or a flame-sterilized, nichrome wire. Put the loop into the positive Lauryl Tryptose tube and immediately into an EC Medium with MUG tube. Do not touch the rim of the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



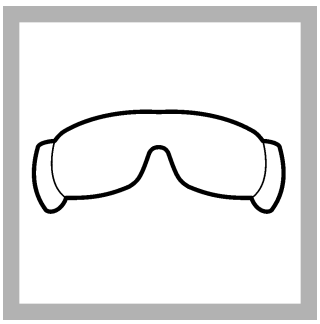
2. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



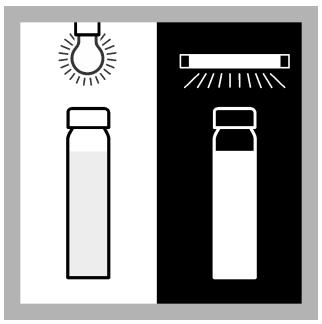
3. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



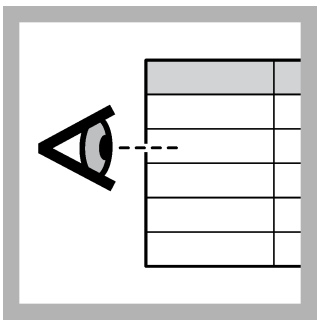
4. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 hours.
Bubbles that form in the inner vials during the first hour are not from bacteria.



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 8 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

Controls for coliform bacteria tests

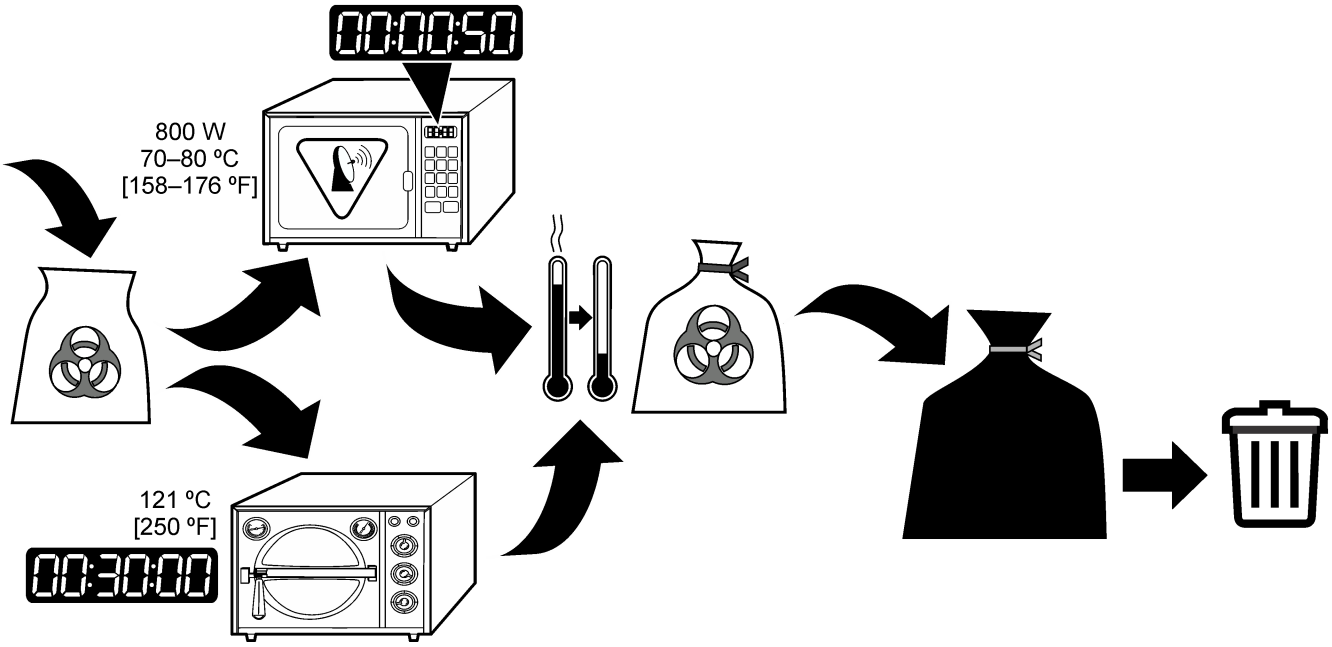
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive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

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1 and the information that follows.

- Microwave—Add 1–2 mL of hypochlorite (bleach) solution to each test container. If a container has a lid, do not close it too tightly. Put the container in the microwave at 70–80 °C (158–176 °F) for 50 seconds. Wait 10 to 15 minutes. Pour the liquid down the drain.
- Autoclave—Put the used test containers in a contaminated items bag or biohazard bag to prevent leaks. Do not seal the bag. Put the bag in the autoclave at 121 °C (250 °F) for 30 minutes at 1.0 bar (15 psi) of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

Figure 1 Bacteria disposal



Summary of method

The Most Probable Number (MPN) method, which is also referred to as the Multiple Tube Fermentation (MTF) technique, uses screw-capped tubes that contain sterile broth medium. The tubes contain an inverted inner vial (a Durham tube) for gas collection. Sample is 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)	10	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 without Durham tubes (*E. coli* confirmation)	varies	15/pkg	2471515
EC Medium with MUG broth tubes with Durham tubes (fecal coliform and *E. coli* confirmation)	varies	15/pkg	2282415

Required apparatus

Description	Quantity/Test	Unit	Item no.
Alcohol burner	1	each	2087742
Sampling bags, Whirl-Pak® without dechlorinating agent, 207 mL	1	100/pkg	2233199
Dri-bath incubator, 12 well	1	each	2281400
Inoculating loop, nichrome wire	varies	each	2112100
UV lamp, long-wave, 115 VAC	1	each	2184300

Required apparatus (continued)

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

Optional media and reagents

Description	Unit	Item no.
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dilution water, buffered, 99 mL, sterile	25/pkg	1430598
Powder pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166

Optional reagents and apparatus

Description	Unit	Item no.
Biohazard bag	200/pkg	2463300
Sampling bags, Whirl-Pak® without dechlorinating agent, 207 mL	500/pkg	2233100
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700
E. coli fluorescence standard	each	2361100
Inoculating loops, sterile, disposable	25/pkg	2749125
Isopropyl alcohol	500 mL	1445949
UV lamp, long-wave, portable, 4 watt	each	2415200
Laboratory marker	each	2092000
Pipet, serological, 1 mL, sterile, disposable, individually wrapped	50/pkg	2092835
Pipet, serological, 10 mL, sterile, disposable, individually wrapped	50/pkg	2092628
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Pipet Aid, 110 VAC recharger, four replacement filters (UL, CSA approved)	each	2551701
Wicks, replacement, for alcohol burner (2087742)	10/pkg	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to 1 L of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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