Coliforms, Presence/Absence

DOC316.53.01191

Presence/Absence (P/A) Method **Method 8319 and Method 8364**

P/A Broth (Method 8319) **Ampules or disposable bottles**

P/A Broth with MUG (Method 8364)

Scope and application: For water and wastewater.

Test preparation

Before starting

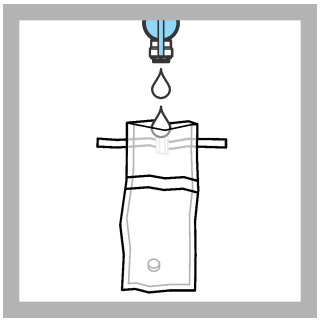
The broths for both methods in this procedure change color when the test result is positive. The P/A Broth with MUG fluoresces under a UV light when *E. coli* is in the sample culture.
Set the temperature of the incubator to 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.
Wash hands thoroughly with soap and water.
Make sure that all of the materials that come in contact with samples are sterile.
Use sterilized Whirl-Pak® bags or bottles for sample collection. If the sample has been disinfected, use bags or bottles that contain a dechlorinating agent.

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 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. 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 immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

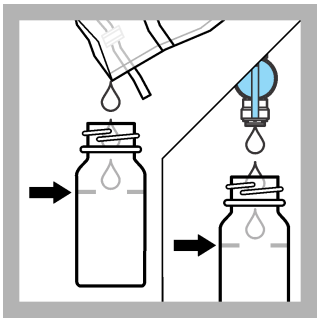
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P/A procedure with bottles



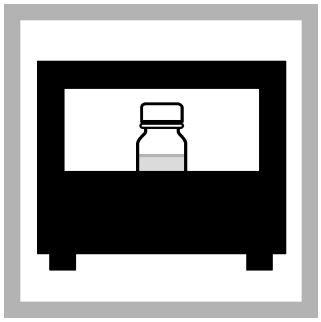
1. Collect 100 mL of sample in a sterile container. Use aseptic technique to prevent contamination.

Note: *If the sample has been disinfected, use a container that contains a dechlorinating agent.*

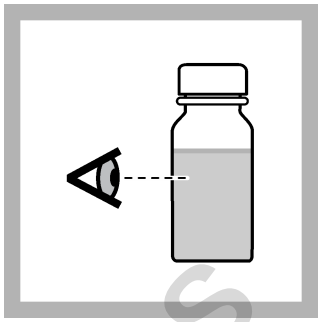


2. Add the sample to the fill line of a P/A bottle.

Note: *Samples that have not been disinfected can be added directly from a faucet or spigot.*

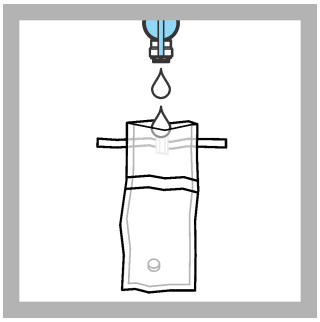


3. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 hours.



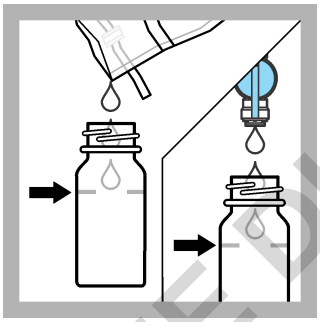
4. After 24 hours, look for a color change. If there is no color change, incubate the sample for an additional 24 hours. If there is no color change after 48 hours, the test result is negative. Refer to Summary of method on page 5.

P/A procedure with ampules



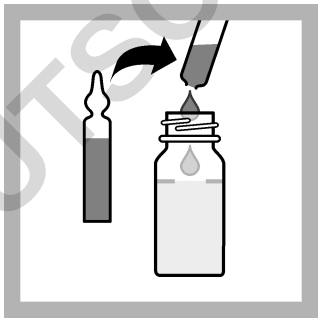
1. Collect 100 mL of sample in a sterile container. Use aseptic technique to prevent contamination.

Note: *If the sample has been disinfected, use a container that contains a dechlorinating agent.*

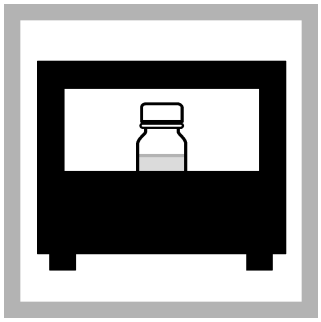


2. Add the sample to the fill line of a sterilized sampling bottle.

Note: *Samples that have not been disinfected can be added directly from a faucet or spigot.*

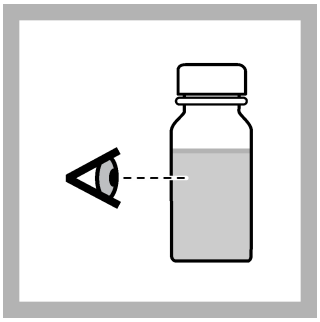


3. Use an ampule breaker to aseptically open a broth ampule. Add the contents of the ampule to the bottle.



4. Incubate the sample at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 hours.

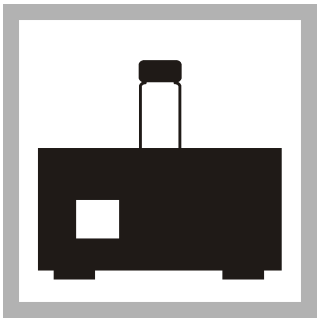
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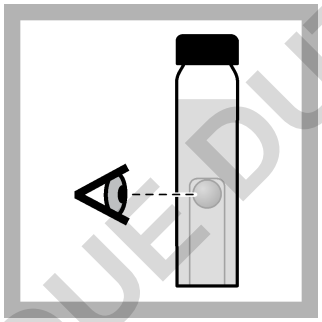
5. After 24 hours, look for a color change. If there is no color change, incubate the sample for an additional 24 hours. If there is no color change after 48 hours, the test result is negative. Refer to Summary of method on page 5.

Confirmation procedure

All presumptive positive test results must be validated with a confirmation media. The confirmation media prevents the growth of non-target organisms and helps the growth of the target organisms. If the media for the presumptive procedure contains MUG, use the UV confirmation procedure on page 4.



1. Incubate the inoculated confirmation media at the specified temperature for the specified time.



2. After the incubation period, look for gas formation in the inner tube and turbidity. A positive reaction is confirmation that coliform bacteria is in the sample.

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Table 1 Confirmation options

Bacteria	Confirmation media	Incubation	Positive result
Total coliform	Brilliant Green Bile broth	24 to 48[1] hours at 35 ±0.5 °C	Gas and turbidity
Fecal coliform	EC Medium tubes	24 hours at 44.5 ±0.2 °C	Gas and turbidity
E. coli	EC Medium with MUG tubes	24 hours at 44.5 ±0.2 °C	UV fluorescence (refer to UV confirmation procedure on page 4)

[1] Examine after 24 hours. If no reaction is seen, incubate for an additional 24 hours.

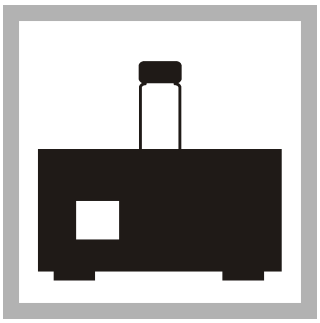
UV confirmation procedure

⚠CAUTION

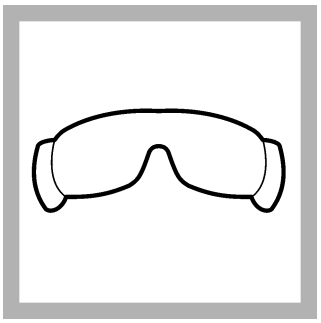
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

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.

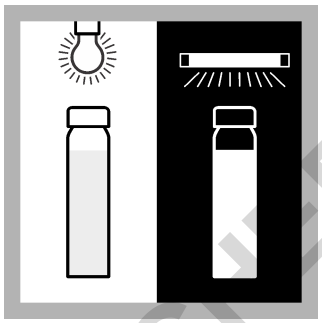
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



1. Incubate the inoculated confirmation media at the specified temperature for the specified time.



2. Put on UV safety goggles



3. Illuminate the incubated sample that contains MUG broth with a long-wave UV lamp.
If the sample fluoresces, *E.coli* bacteria is confirmed to be in the sample.

Controls for coliform bacteria tests

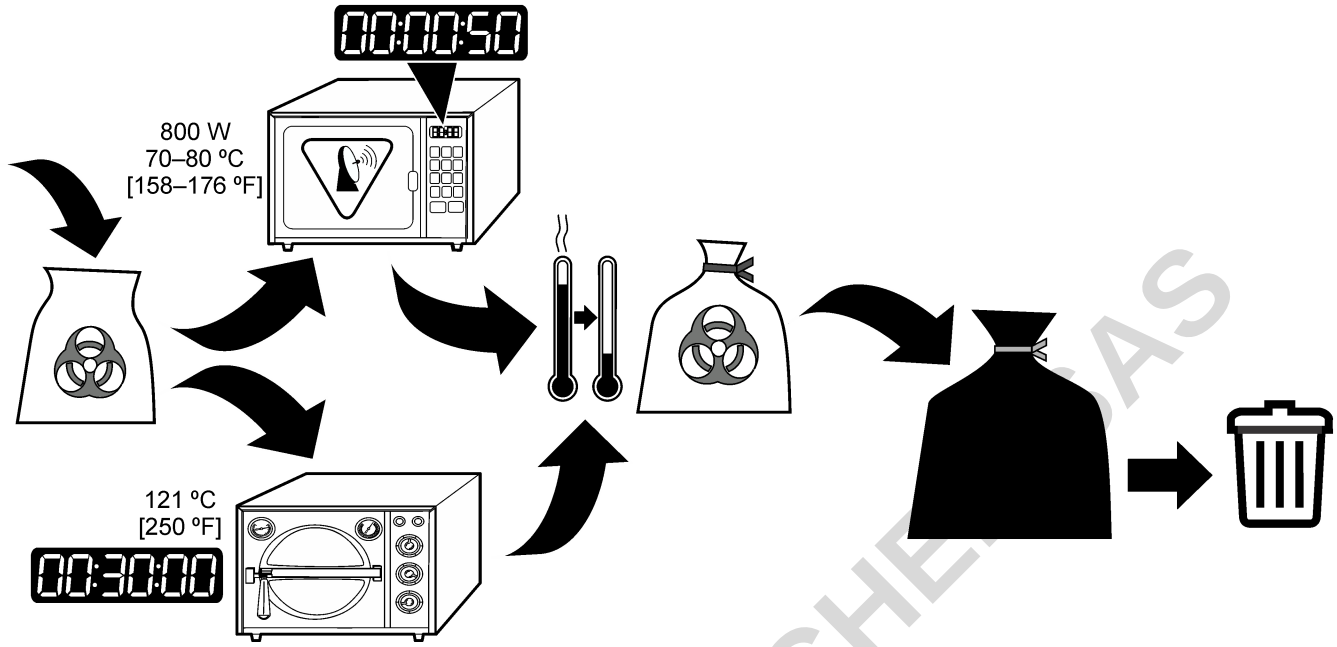
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive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

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1.
Hypochlorite (bleach) solution can also be used. Add 1–2 mL of hypochlorite (bleach) solution to each test container. Wait 10 to 15 minutes. Pour the liquid down the drain.

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Figure 1 Bacteria disposal



Summary of method

The P/A Broth contains lactose and lauryl tryptose with bromcresol purple indicator. When coliform bacteria are in the sample, the bacteria ferment the lactose in the broth during incubation and acidify the sample. The acidity causes the color of the indicator to change from purple to yellow. Positive results must be confirmed with confirmation media. Coliform bacteria will cause gas and turbidity to form in the confirmation media.

The P/A Broth with MUG contains MUG reagent (4-methylumbelliferyl-b-D-glucuronide) in addition to the lactose and lauryl tryptose broths with bromcresol purple. The MUG reagent produces a fluorogenic product when hydrolyzed by glucuronidase (an enzyme specific to E. coli). MUG reacts with non gas-producing (anaerogenic) strains of E. coli and works well when competitive organisms are in the sample. A positive fluorescing result is confirmation of both total coliform bacteria and *E. coli*.

Consumables and replacement items

Presence/Absence broth

Description	Quantity/test	Unit	Item no.
Presence/Absence Broth, 20-mL ampules	1	25/pkg	2494925
Presence-Absence Broth, disposable bottles	1	12/pkg	2323212
Presence-Absence Broth, disposable bottles	1	50/pkg	2323250
Presence/Absence Broth with MUG, 20-mL ampules	1	25/pkg	2495525
Presence/Absence Broth with MUG, disposable bottles	1	12/pkg	2401612
Presence/Absence Broth with MUG, disposable bottles	1	50/pkg	2401650

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006

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Sample collection (continued)

Description	Unit	Item no.
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

Confirmation

Description	Unit	Item no.
Brilliant Green Bile broth tubes (for total coliform confirmation)	15/pkg	32215
EC medium broth tubes (for fecal coliform confirmation)	15/pkg	1410415
EC medium with MUG broth tubes (for *E. coli* confirmation)	15/pkg	2471515
Inoculating loop, nichrome wire	12/pkg	2112100
Inoculating loop, plastic disposable	25/pkg	2749125

UV confirmation

Description	Quantity/Test	Unit	Item no.
UV lamp, long-wave, portable, 4 watt	1	each	2415200
Replacement bulb for portable UV lamp	1	each	2584600
UV lamp, long-wave, 115 VAC	1	each	2184300
UV lamp, long-wave, 230 VAC	1	each	2184302
UV blocking eyewear	1	each	SM730-1033
E. coli fluorescence standard	—	each	2361100

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Incubator rack for 19-mm MPN tubes, holds 25 tubes	each	221500
Laboratory incubator, water bath, 110 VAC	each	2616300
Laboratory incubator, water bath, 220 VAC	each	2616302
Dri-bath incubator, 12 well	each	2281400
Portable incubator with 12 VDC power socket	each	2569900
Portable incubator rack, general purpose/petri dish	each	2580502
Portable incubator rack, MPN tubes, holds 39 tubes	each	2580501
Portable incubator rack, P/A bottles, holds 6 bottles	each	2580500
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
AC power supply for portable incubator, 110–240 VAC	each	2968100

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Optional items

Description	Unit	Item no.
Alcohol burner	Each	2087742
Ampule breaker, 20-mL glass ampules	Each	2564000
Ampule breaker, 2-mL glass ampules	Each	2484600
Bunsen burner	Each	2162700
Replacement wicks for alcohol burner	10/pkg	2097810
Isopropyl alcohol	500 mL	1445949
Sterilization indicator ampules, Sterikon	15/pkg	2811115
Laboratory marker	Each	2092000
Biohazard bag	200/pkg	2463300

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