

Bacteria, Hydrogen Sulfide Producing

DOC316.53.01196

Most Probable Number (MPN) Method

Method 10032

PathoScreen™ Medium

Scope and application: For the detection of Salmonella, Citrobacter, Proteus, Edwardsiella and Klebsiella (some spp.) in drinking water, surface water and recreational water.



Test preparation

Before starting

Wash hands thoroughly with soap and water.

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

Incubate samples for 24–48 hours between 25–35 °C (77–95 °F). The recommended temperature for samples is 30 °C (80 °F).

PathoScreen Medium has a detection sensitivity of 1 CFU/100 mL.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

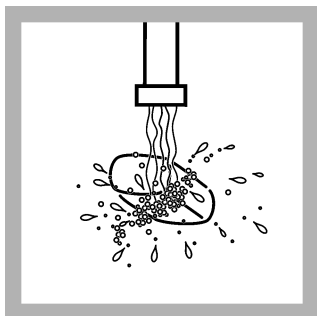
Description	Quantity
Alcohol, ethanol or methanol	100 mL
Bottle, autoclavable	1
Bottle, sample, sterilized, 100-mL fill-to line	1
Clippers, large	1
Dilution water, buffered, 99 mL, sterile	1
Incubator	1
Pipet, serological, 10–11 mL, sterile	1
PathoScreen Medium, MPN Powder Pillows	1
Dechlorinating Reagent Powder Pillows	1
Tube rack	1

Refer to [Consumables and replacement parts](#) on page 5 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. If a Whirl-Pak bag is used, whirl the bag to close and then fold the wire tabs to make a seal. To prevent leaks, do not twist the wire tabs.
- Write the sample information on the container and start the analysis as soon as possible.
- 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 before sample is collected. 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.
- 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.

Test procedure



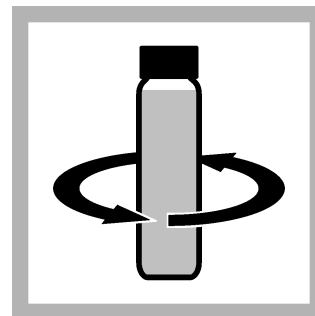
1. Wash hands thoroughly with soap and water.



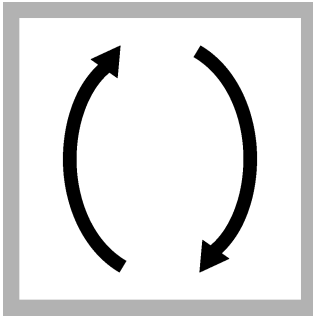
2. Remove the caps from five sterile tubes, one at a time. Use a sterile pipet to add 20 mL of sample into each of the tubes. Use aseptic technique to prevent contamination of the tubes or caps.



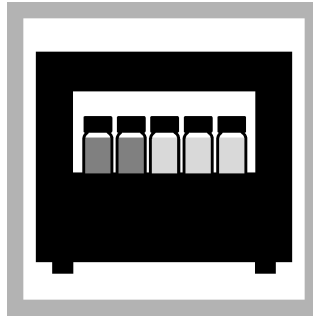
3. Before use, clean the end of the PathoScreen Medium P/A Powder Pillow with alcohol and a cotton swab. Use clippers to aseptically cut the powder pillow open. Add the contents of one powder pillow to each 20-mL tube of sample.



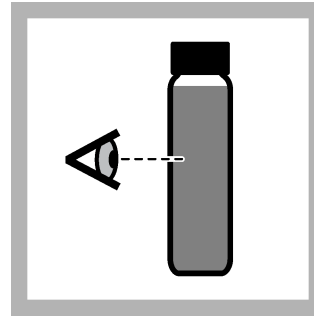
4. Put the caps on the tubes. Immediately swirl each tube to mix.



5. Invert the tubes 2–3 times to thoroughly mix the sample with the medium. The sample color changes to yellow.



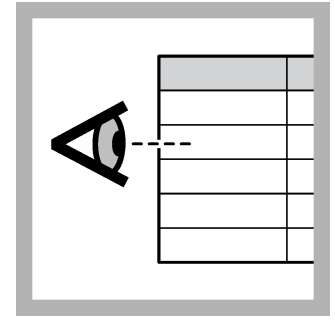
6. Incubate the sample at $30 \pm 0.5 \text{ }^\circ\text{C}$ ($86 \pm 0.9 \text{ }^\circ\text{F}$) for 24–48 hours.
Note: *If an incubator is not available, make sure that the sample is in a location with a constant temperature between $25\text{--}30 \text{ }^\circ\text{C}$ ($77\text{--}86 \text{ }^\circ\text{F}$) for 24–48 hours.*



7. After 24 hours, examine and record the reaction.
Note: *If the temperature is very unstable, incubate the sample for an additional 24 hours.*

If the color changes from yellow to black or if a black precipitate forms, the sample is positive for hydrogen sulfide-producing bacteria.

If there is no change, incubate for an additional 12–24 hours and examine the sample again. If there is no color change, the sample is negative for hydrogen sulfide-producing bacteria.



8. Record the results. Refer to [Table 1](#) on page 3 to find the MPN values.

MPN values

With statistical methods, it is possible to estimate the number of organisms from any combination of positive and negative test results. [Table 1](#) shows the MPN values that are based on the 20 mL of undiluted sample in each of the five tubes. If the sample is diluted, multiply the result by the dilution factor. Refer to [Sample dilution](#) on page 4 to make serial dilutions of the sample.

Example 1: Five tubes of undiluted sample are inoculated. Three of the five tubes are positive. [Table 1](#) shows that the result is 4.6 organisms/100 mL.

Example 2: A river water sample is collected and diluted. A dilution factor of 10,000 is prepared and five tubes are inoculated. Two of the five tubes are positive. [Table 1](#) shows that the result is 2.6. To find the result: $2.6 \times 10,000 = 26,000$. Record the result as 26,000.

Table 1 Five-tube MPN values

Number of positive tubes	MPN for each 100 mL
0	< 1.1
1	1.1
2	2.6
3	4.6
4	8.0
5	> 8.0

Dilution for MPN testing

The MPN method can be used for drinking water, marine and fresh recreational waters, swimming pools, lakes, shellfish-growing waters, heavily polluted waters and wastewater.

Refer to [Sample dilution](#) on page 4 to dilute heavily contaminated waters with the serial dilution procedure.

Sample dilution

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

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

1. Wash hands thoroughly with soap and water. Gloves are optional.
2. Vigorously mix the sample for 30 seconds.
3. Open a bottle of sterile buffered dilution water.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert for 30 seconds (25 times). This is a 10-fold dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. Continue to make dilutions until there are three bottles that contain the dilutions listed in [Table 2](#).

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

Table 2 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

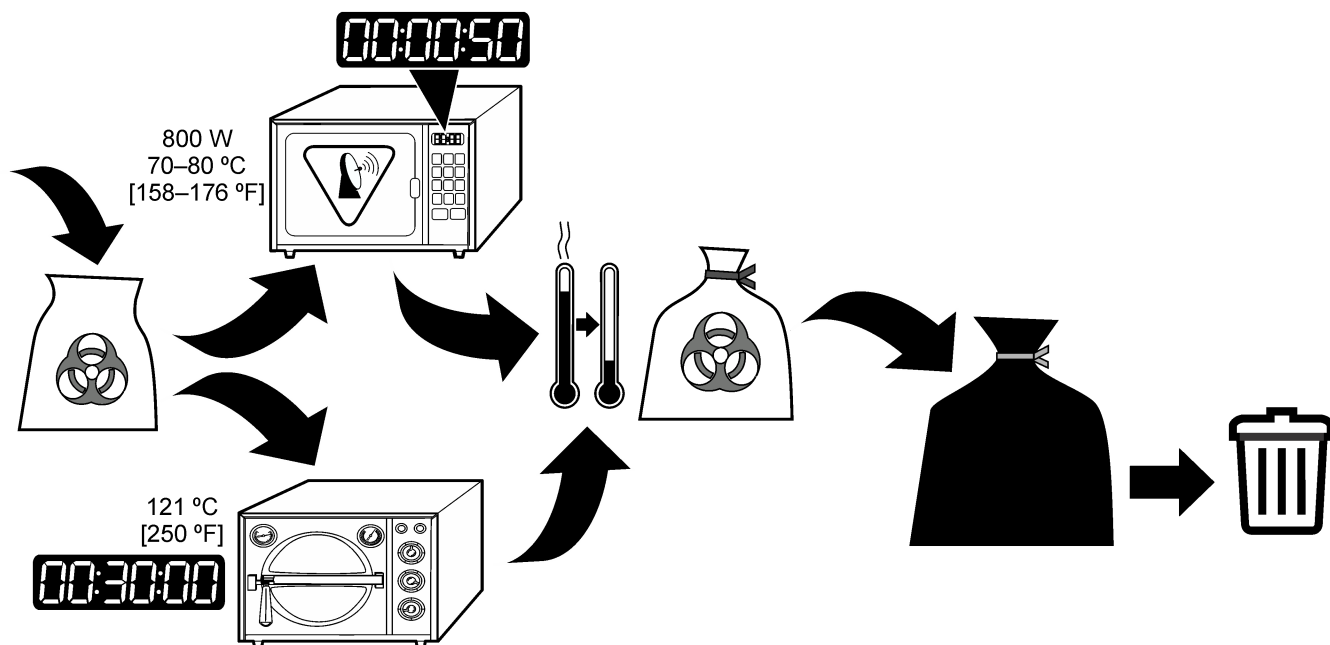
Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to [Figure 1](#) to sterilize with a microwave or an autoclave.

Use one of the methods that follow to kill the cultured bacteria before disposal:

- Hypochlorite (bleach) solution can also be used. 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.
- Kill the cultured bacteria with autoclave pressure. 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 15 lb 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

PathoScreen Medium determines if hydrogen sulfide-producing bacteria (e.g., Salmonella, Citrobacter, Proteus, Edwardsiella and some species of Klebsiella) are in the sample. The sterilized powder is a reliable medium to monitor drinking water systems in developing tropical countries, remote field locations and disaster or emergency situations. PathoScreen Medium is dehydrated, sterilized and packaged in powder pillows. The powder pillows are available for both Presence/Absence (P/A) and Most Probable Number (MPN) methods. Each powder pillow has a sufficient amount of medium for one test.

Consumables and replacement parts

Required media and reagents

Description	Quantity/Test	Unit	Item no.
PathoScreen™ medium, MPN powder pillows, 20-mL sample	1	100/pkg	2610796
Dechlorinating Reagent Powder Pillows	1	100/pkg	1436369
Dilution water, buffered, 99 mL, sterile ¹	1	25/pkg	1430598

Required apparatus

Description	Quantity/Test	Unit	Item no.
Autoclave, 120 VAC	1	each	2898600
Biohazard bag	1	200/pkg	2463300
Sampling bags, Whirl-Pak® without dechlorinating agent, 180 mL	1	100/pkg	2075333
Bottle, autoclavable, 125 mL	1	6/pkg	2324333
Bottle, autoclavable, 125 mL	1	48/pkg	2324373
Clippers, large	1	each	2065800
Bottle, sample, sterilized, 100-mL fill-to line, disposable	1	12/pkg	2495012

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

Required apparatus (continued)

Description	Quantity/Test	Unit	Item no.
Bottle, sample, sterilized, 100-mL fill-to line, disposable	1	50/pkg	2495050
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	1	12/pkg	2599112
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	1	50/pkg	2599150
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, serological, 10 mL, sterile, disposable, individually wrapped	1	50/pkg	2092628
Pipet filler, 110 VAC recharger, four replacement filters (UL, CSA approved)	1	each	2551701
Rack, coliform tube	1	each	221500
Vials, MPN	1	10/pkg	1497054

Optional media and reagents

Description	Unit	Item no.
Powder pillows for buffered dilution water (25 of each) ²	50/pkg	2143166
Peptone Powder Pillows, 1 g	30/pkg	2142964

Optional reagents and apparatus

Description	Unit	Item no.
Dri-bath incubator, 12 well	each	2281400
Pipet, TenSette [®] , 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Pipet filler, safety bulb	each	1465100
Sampling bags, Whirl-Pak [®] without dechlorinating agent, 207 mL	100/pkg	2233199
Sampling bags, Whirl-Pak [®] without dechlorinating agent, 720 mL	10/pkg	1437297
Sterilization Indicator, Sterikon [®]	15/pkg	2811115
Sterilization Indicator, Sterikon [®]	100/pkg	2811199

² 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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