Bacteria, Hydrogen Sulfide Producing

Methods 8506 and 10032

Most Probable Number (MPN) and Presence/ Absence (P/A) Methods

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

PathoScreen Medium

Hach's PathoScreen Medium detects the presence of hydrogen sulfide-producing bacteria including *Salmonella, Citrobacter, Proteus, Edwardsiella,* and some species of *Klebsiella.* The sterilized powder medium is easy to use and produces easy-to-interpret results. This reliable, inexpensive medium is well suited for monitoring drinking water systems in developing tropical countries, in remote field locations, and in disaster or emergency situations.

Convenient Packaging

PathoScreen Medium is dehydrated, sterilized, and packaged in powder pillows. Powder pillows are available for both Presence / Absence (P / A) and Most Probable Number (MPN) testing. Each powder pillow contains enough medium for one test. The medium is shipped with a Certificate of Analysis and has an expiration date printed on the label.

For P / A testing, add one P / A powder pillow to a 100-mL sample. For MPN testing, add one MPN Pillow to a 20-mL sample. For MPN testing, you will need to inoculate a set of five tubes.

Tips and Techniques

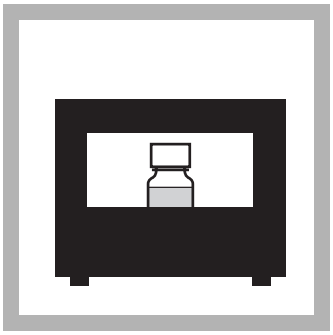
- Incubate samples 24–48 hours between 25–35 °C, 77–95 °F. (30 °C, 80 °F is considered optimal.)
- PathoScreen Medium has a detection sensitivity of 1 CFU/100 mL.

Using PathoScreen Medium P/A Pillows Method 8506

1. Wash your hands thoroughly with soap and water.

2. Collect 100 mL of sample in a sterile sample container. (See *Collecting and Preserving Samples on page 1059* for details.)

3. Swab the end of the PathoScreen Medium P / A Pillow with alcohol and aseptically cut it open with clippers. Add pillow contents to the 100 mL sample.

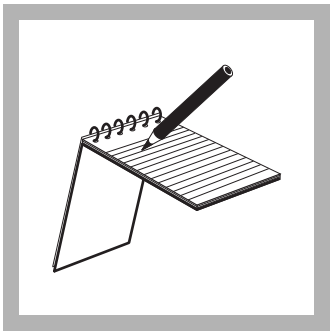


4. Place the bottle in a location with a constant temperature between 25–35 °C for 24–48 hours.

Note: *If an incubator is available, incubate the sample at 30 ±0.5 °C for 24 to 48 hours.*

5. Note the reaction after 24 hours of incubation.

Note: *If the temperature varies significantly, incubation may be extended an additional day.*



6. Record results. (See *Table 1*.)

7. Dispose of all completed tests appropriately. (See *Disposing of Completed Tests on page 1060*.)

Table 1 Interpreting P/A Results

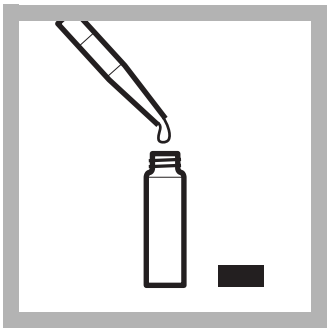
Hydrogen sulfide producing bacteria			
Test Results	**Positive**	**Negative**	**Follow-up**
Color changes from yellow to black	X		
Black precipitate forms	X		
No color change		X	Incubate additional 12–24 hours and re-evaluate. If there is no color change, record as negative.

Conducting MPN Tests with PathoScreen Medium

The MPN method can be used for drinking water, as well as marine and fresh recreational waters, swimming pools, lakes, shellfish-growing waters, heavily polluted waters, and wastewater. For water that is heavily contaminated, use the multiple tube decimal dilution procedure.

Using PathoScreen Medium MPN Pillows — Method 10032

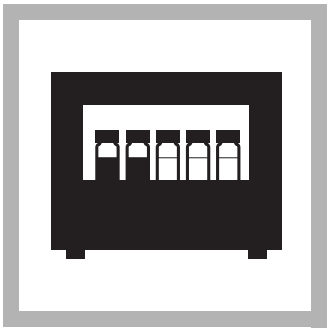
1. Wash your hands thoroughly with soap and water.



2. Remove the caps from five sterile tubes one at a time and pipet 20 mL of sample into each of the tubes with a sterile pipet. Use aseptic technique to avoid contaminating the tubes or the caps.

3. Swab the end of a PathoScreen Medium MPN Pillow with alcohol and aseptically cut it open with clippers. Add pillow contents to the 20 mL sample.

4. Cap each tube immediately. Invert the tubes a few times to thoroughly mix the sample with the medium.

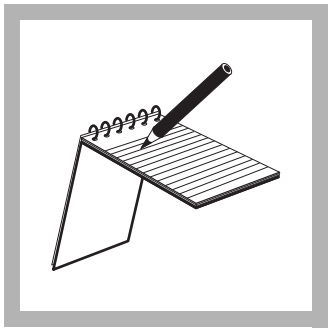


5. Place the tubes in a location with a constant temperature of 25–35 °C for 24–48 hours.

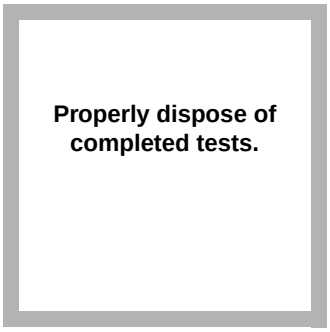
Note: *If an incubator is available, incubate the sample at 30 ±0.5 °C for 24–48 hours.*

6. Note the reaction after 24 hours of incubation. (See *Table 2* on page *1106*.)

Note: *If the temperature varies significantly, continue to incubate negative tubes for an additional day.*



7. Record results. (See *Table 3* on page *1106*.)



8. Dispose of all completed tests appropriately. (See *Disposing of Completed Tests on page 1060*.)

Table 2 Interpreting MPN Results

Hydrogen sulfide producing bacteria			
Test Results	**Positive**	**Negative**	**Follow-up**
Color changes from yellow to black	X		
Black precipitate forms	X		
No color change		X	Incubate additional 12–24 hours and re-evaluate. If there is no color change, record as negative.

Using statistical methods it is possible to estimate the number of organisms from any combination of positive and negative test results. The MPN values in *Table 3* are based on 20 mL of undiluted sample in each of five tubes. If the sample is diluted, multiply the result by the dilution factor.

Example 1: Five tubes of undiluted sample are inoculated. Positive results are obtained from three of the five tubes. The result obtained from *Table 3* is 4.6.

Example 2: A river water sample is collected and diluted. A dilution factor of 10,000 is prepared and five tubes are inoculated. Positive results are obtained from two of the five tubes. The result obtained from two of the five tubes. The result obtained from *Table 3* is 2.6. This result is multiplied by 10,000, and a result of 26,000 is recorded.

Table 3 Five-tube MPN values for undiluted, 20-mL samples (95% confidence limits)

Positive Tubes	MPN/100 mL
0	<1.1
1	1.1
2	2.6
3	4.6
4	8.0
5	>8.0

Media, Reagents and Apparatus

Medium

Description	Unit	Cat. No.
PathoScreen™ Medium, P / A Pillows, 100-mL sample	50 / pkg	26106-96
PathoScreen™ Medium, MPN Pillows, 20-mL sample	50 / pkg	26107-96

For Dilution Water

Bottle, polysulfone, autoclavable (for preparing buffered dilution water)	12 / pkg	22453-00
Buffered Dilution Water, sterile, 99-mL*	25 / pkg	14305-98
Dechlorinating Reagent Powder Pillows	100 / pkg	14363-69
Magnesium Chloride and Potassium Dihydrogen Phosphate Powder Pillows	25 of each	21431-66
Peptone Powder Pillows, 1-g	30 / pkg	21429-64
Pipet, sterile, disposable, 11-mL	25 / pkg	2097-98
Pipet, sterile, disposable, individually wrapped, 10-mL	50 / pkg	20926-28
Pipet, sterile, disposable, 10-mL	50 / pkg	20928-28
Pipet Filler, portable, with recharger (UL, CSA approved), 110 VAC	each	25517-01

Apparatus

Alcohol Burner, 100-mL	each	20877-42
Autoclave, Automatic, 120 VAC	each	24630-00
Autoclave, Automatic, 240 VAC	each	24630-02
Clippers, large	each	20658-00
Contaminated Items Bags	200 / pkg	24633-00
Germicidal Cloth	50 / pkg	24632-00
Incubator, Culture, 120 VAC	each	26192-00
Incubator, Culture, 220 VAC	each	26192-02
MPN Vials	10 / pkg	14970-54
Rack for coliform tubes	each	24979-03
Sampling Containers		
Sampling Bags, Whirl-Pak with dechlorinating agent, 170-mL	100 / pkg	20753-33
Sampling Bottles, autoclavable	6 / pkg	23243-33
Sampling Bottles, autoclavable	48 / pkg	23243-73
Sampling Bottles, sterilized, 100-mL fill-to line	12 / pkg	24950-12
Sampling Bottles, sterilized, 100-mL fill-to line	50 / pkg	24950-50
Sampling Bottles, sterilized, 100-mL fill-to line, with dechlorinating agent	12 / pkg	25991-12
Sampling Bottles, sterilized, 100-mL fill-to line, with dechlorinating agent	50 / pkg	25991-50

* Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate.

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

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