



# ***ANALYTICAL PROCEDURE***

## **PathoScreen™ Field Kit**

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### **Bacteria: Hydrogen Sulfide Producing**

**Method 10032**

*Scope and Application: For detection of Salmonella, Citrobacter, Proteus, Edwardsiella, and some species of Klebsiella in drinking water, surface water, and recreational waters.*

**Media: PathoScreen\* Medium**

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### **Detecting Bacteria and Pathogens**

Many serious diseases, such as typhoid fever and dysentery, can be traced directly to pathogenic microorganisms in polluted water. These disease-producing organisms are discharged in fecal wastes and are difficult to detect in water supplies. People may come in contact with these pathogens in drinking water or in recreational waters such as swimming pools, rivers, streams, lakes, and bathing beaches.

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\* PathoScreen is a trademark of Hach Company.

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Direct testing for bacterial pathogens is often impractical and requires lengthy and complex test procedures, making the use of *indicator organisms* routine. Indicator organisms are usually of fecal origin. They are present when pathogens are present and absent when pathogens are absent. They are usually not pathogenic.

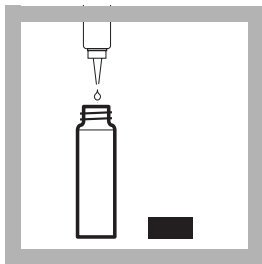
No single organism satisfies all of the criteria for an indicator. For example, in temperate climates total coliform bacteria are commonly used as indicator organisms in potable water supplies. In most tropical climates indigenous *E. coli* produce positive reactions when traditional coliform tests are used. To minimize false positive results, other bacteria known to be associated with fecal contamination may be used as indicator organisms. Hydrogen sulfide-producing bacteria are associated with the presence of fecal contamination and may be used as indicator organisms.

The PathoScreen™ Medium detects the presence of hydrogen sulfide-producing bacteria including *Salmonella*, *Citrobacter*, *Proteus*, *Edwardsiella*, and some species of *Klebsiella*. Indigenous *E. coli* does not interfere with the PathoScreen test, which makes it an excellent alternative to coliform testing. The sterilized powder medium is easy to use and produces easy-to-interpret, reliable results. PathoScreen Medium is well-suited for monitoring drinking water systems in developing tropical countries, in remote field locations, and in disaster or emergency situations.

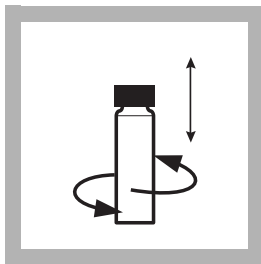
## Preparing Sample Containers

Sample bottles must be sterilized before every use. Clean bottles thoroughly with soap and water, then perform the sterilization procedure. Wash hands thoroughly with soap and water to minimize contamination of sample.

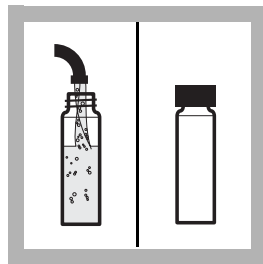
## Sterilization Procedure



**1.** Add 10–12 drops of Bleach Solution to the bottle. Cap.



**2.** Swirl and invert the bottle to completely coat the walls of the bottle and inner surface of the cap. Allow the bottle to sit for 2 minutes.



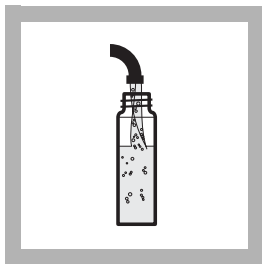
**3.** Rinse the bottle several times with sample water. The bottle is now ready for use in the test.

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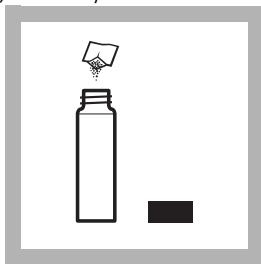
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## Conducting the P-A Test with PathoScreen Medium

**Note:** *Wash hands thoroughly with soap and water to minimize contamination of sample.*

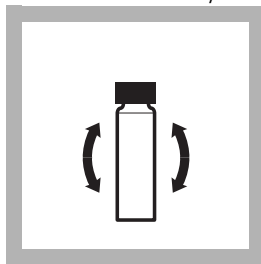


**4.** Fill the sterilized bottle to the shoulder with sample (approximately 20 mL).



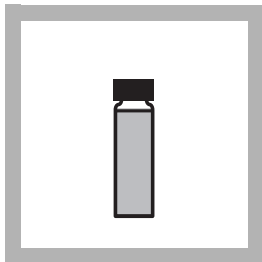
**5.** Add the contents of one PathoScreen Medium powder pillow to the sample.

**Note:** *To avoid contamination, swab the pillow with alcohol before opening.*



**6.** Cap the bottle immediately. Invert to thoroughly mix the sample with medium.

Place the bottle in a location with constant temperature at 25–35 °C (77–95 °F) for 24 to 48 hours.



**7.** Evaluate the reaction after 24 hours ([Table 1](#) ). If temperatures have varied significantly, continue to incubate negative samples for an additional 24 hours.

**Table 1 Interpreting 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 to 24 hours and reevaluate. If there is no color change, record as negative.

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## Conducting the MPN Test with PathoScreen™ Medium

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

### Sample Size and Dilution—Nonpotable Water

Testing nonpotable water samples may require dilution of the original sample, based on the density of organisms. For example, when examining swimming pool water, inoculate five tubes with 20 mL of a 1:10 dilution of sample. Use sterile buffered water for dilutions.

Use statistical methods to estimate the number of organisms from any combination of positive and negative test results. The MPN values ([Table 2](#) ) are based on 20 mL of undiluted sample in each of the 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. Based on [Table 2](#) , the result 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. Based on [Table 2](#) , the result (2.6) is multiplied by 10,000 for a final result of 26,000.

**Table 2 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

## Collecting and Preserving Samples

### General Guidelines

Use proper sampling techniques to ensure seasonal variances are detected and the results are representative of the sample source. Avoid sample contamination during collection. Carefully open each sample container just prior to collection and close immediately following collection. Do not lay the cap down or touch the mouth or inside of the container.

The PathoScreen medium contains sodium thiosulfate, so no dechlorination is necessary if the sample is added directly to the medium on site. Otherwise, treat samples to destroy chlorine residual and transport for analysis immediately after collection. Sodium thiosulfate sterilized within the collection container is commonly used to destroy chlorine residual.

If samples cannot be analyzed immediately, label and store samples for up to 24 hours. In warm climates, maintain sample temperature between 4–10 °C (39–50 °F). Failure to properly collect and transport samples will cause inaccurate results.



## **Collecting from Faucets, Spigots, Hydrants, or Pumps**

- Collect representative samples by allowing the water to run at a moderate rate (without splashing) for 2–3 minutes before sampling.
- Do not adjust rate of flow during sample collection.
- Avoid valves, spigots, or faucets that swivel or leak.
- Remove aerators or screens before sample collection.

## **Collecting from Rivers, Lakes, and Reservoirs**

- Do not sample near the edge or bank.
- Remove the cap, hold the sample container near the bottom, and plunge the container mouth-down into the water. (This technique excludes surface scum.)
- While submerged, position the mouth of the container into the current.
- In non-flowing water, tilt the mouth of the container up slightly and allow it to fill slowly.

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## Reorder Reagents and Parts

Bleach Solution, 5 to 6%.....	59 mL.....	28592-26
Buffer Dilution Water.....	25/pkg.....	14305-98
PathoScreen Medium Powder Pillows.....	100/pkg.....	26107-96
PathoScreen Medium Manual .....	each.....	28591-88
Sample Cells, with Cap.....	6/pkg.....	24276-06