

Presence-Absence Broth

Intended Use

Presence-Absence Broth is used for detecting coliforms in treated water.

Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection test for coliforms in water. The test is a simple modification of the multiple-tube procedure.¹ One test sample, 100 mL, is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms based on the presence or absence of lactose fermentation.¹ This test is based on the principle that coliforms and other pollution indicator organisms should not be present in a 100 mL water sample.²⁻⁸

Comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.¹ The P-A test is described in standard methods for water testing¹ and by U.S. Environmental Protection Agency.⁹

Principles of the Procedure

Beef extract and peptones provide the nitrogen, vitamins and amino acids in Presence-Absence Broth. Lactose is the carbon source in the formula. The potassium phosphates provide buffering capacity; sodium chloride provides essential ions. Sodium lauryl sulfate is the selective agent, inhibiting many organisms except coliforms. Bromocresol purple is used as an indicator dye; lactose-fermenting organisms turn the medium from purple to yellow with or without gas production.

Formula

Difco™ Presence-Absence Broth

Approximate Formula* Per Liter	
Beef Extract.....	3.0 g
Peptone.....	5.0 g
Lactose.....	7.46 g
Pancreatic Digest of Casein.....	5.90 g
Proteose Peptone No. 3.....	3.93 g
Dipotassium Phosphate.....	1.35 g
Monopotassium Phosphate.....	1.35 g
Sodium Chloride.....	2.46 g
Sodium Lauryl Sulfate.....	0.05 g
Bromocresol Purple.....	8.5 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 91.5 g of the powder in 1 L of purified water to prepare at triple strength. Mix thoroughly.
2. Warm gently to completely dissolve the powder.
3. Dispense 50 mL amounts into screw cap 250 mL milk dilution bottles.
4. Autoclave at 121°C for 12 minutes, with total autoclave time not to exceed 30 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Presence-Absence Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.05% solution, soluble in purified water. Solution is purple, clear to very slightly opalescent.
Prepared Appearance:	Purple, clear to very slightly opalescent, without significant precipitate.
Reaction of 3.05% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Presence-Absence Broth

Prepare Presence-Absence Broth in triple strength solution (9.15%). Sterilize in 50 mL quantities in milk dilution bottles with capacity greater than 150 mL. Add 100 mL of drinking water after medium is sterilized and cooled to room temperature. Inoculate bottles with the test organisms. Incubate bottles at 35 ± 0.5°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR CHANGE
<i>Enterococcus faecalis</i>	29212	10 ² -10 ³	Partial inhibition to fair	No change to slight yellow
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good	Yellow with or without gas production
<i>Escherichia coli</i>	13762	10 ² -10 ³	Good	Yellow with or without gas production
<i>Pseudomonas aeruginosa</i>	27853	10 ² -10 ³	Partial inhibition to fair	No change



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Procedure

1. Collect water samples as described in recommended procedures.^{1,9}
2. Inoculate 50 mL of the sterile triple strength P-A Broth with 100 mL of the water sample.
3. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample.
4. Incubate at 35 ± 0.5°C.
5. Inspect for acid and gas production after 24 and 48 hours of incubation.

Expected Results

A distinct yellow color indicates lactose fermentation, an acid reaction. Gas production can be observed by a foaming reaction when the bottle is gently shaken. Any amount of gas and/or acid is a positive presumptive test requiring confirmation.¹ Report results as positive or negative for coliforms per 100 mL of sample.

Confirmation and differentiation of coliforms detected by the P-A test may be achieved by use of appropriate confirmatory media, incubation times and temperatures as outlined in appropriate references.^{1,9}

Limitations of the Procedure

1. The P-A test is only a presumptive test for coliforms.
2. Extending the P-A test incubation period to 72 or 96 hours will allow isolation of other indicator organisms. However, indicator bacteria isolated after 48 hours incubation may not be considered for regulatory purposes.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Weiss and Hunter. 1939. J. Am. Water Works Assoc. 31:707.
3. Clark. 1968. Can. J. Microbiol. 14:13.
4. Clark. 1969. Can. J. Microbiol. 15:771.
5. Clark and Vlassoff. 1973. Health Lab. Sci. 10:163.
6. Clark and Pagel. 1977. Can. J. Microbiol. 23:465.
7. Clark. 1980. Can. J. Microbiol. 26:827.
8. Clark, Burger and Sabatinos. 1982. Can. J. Microbiol. 28:1002.
9. Federal Register. 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and *E. coli*). Fed. Regist. 54:27544.

Availability

Difco™ Presence-Absence Broth

CCAM EPA SMWW

Cat. No.	219200	Dehydrated – 500 g
	219100	Dehydrated – 2 kg