Presence Absence Broth
SKU: 700003320, 700003321, 700003322, 700003323
NCM0122

Intended Use
Presence Absence Broth is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test in a laboratory setting. Presence Absence Broth is not intended for use in the diagnosis of disease or other conditions in humans.

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

Comparative studies with the membrane filter procedure indicate the PA test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection. The PA test is described in standard methods for water testing and U.S. EPA.

Typical Formulation

Beef Extract	3.0 g/L
Enzymatic Digest of Gelatin	5.0 g/L
Lactose	7.46 g/L
Enzymatic Digest of Casein	9.83 g/L
Dipotassium Phosphate	1.35 g/L
Monopotassium Phosphate	1.35 g/L
Sodium Chloride	2.46 g/L
Sodium Lauryl Sulfate	0.05 g/L
Bromocresol Purple	0.0085 g/L

Final pH: 6.8 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Prepare triple strength concentration by adding 91.5 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Dispense 50 mL into a 250 mL screw-cap milk dilution bottle.
4. Autoclave at 121°C for 12 minutes.
5. Cool and add 100 mL water sample.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear, very dark reddish purple, and none to light precipitate.

Expected Cultural Response: Cultural response in Presence Absence Broth incubated aerobically at 35 ± 2 °C and examined for growth after 18 - 48 hours.

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Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 29212	10 - 300	Good to excellent	No change (purple to light grey-yellow)
Escherichia coli ATCC® 25922	10 - 300	Good to excellent	Yellow
Escherichia coli ATCC® 11775	10 - 300	Good to excellent	Yellow
Pseudomonas aeruginosa ATCC® 27853	10 - 300	Partial inhibition	No change (purple to grey-purple)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate 50 mL of the sterile triple strength PA Broth with 100 mL of the water sample.
2. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample. Incubate at 35 ± 0.5°C.
3. Inspect for acid and gas production after 24 and 48 hours incubation.

Results

An acid reaction from lactose fermentation is indicated by a distinct yellow color in the medium. Gas production is indicated by bubbles or foam present in the medium. Any amount of gas and/or acid is a positive presumptive test requiring confirmation. Report results as positive or negative for coliforms per 100 mL of sample.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

1. The PA test is only a presumptive test for coliforms.
2. Confirmation and differentiation of coliforms detected by the PA test may be achieved through biochemical testing, incubation time, and temperatures as outlined in appropriate references.
3. Extending PA 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.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 2017. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C.
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3. Clark, J. A. 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. Can. J. Microbiol. 26:827-832.
4. Clark, J. A., C. A. Burger, and L. E. Sabatinos. 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can. J. Microbiol. 28:1002-1013.
5. Federal Register. 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and *E. coli*). Fed Regist. 54:27544-27568.

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