PRESENCE-ABSENCE BROTH (7500)

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.

Product Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection for coliforms in water. The test is a simple modification of the multiple-tube procedure.[1] 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.[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.[2-4]

Comparative studies with the membrane filter procedure indicate 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 testing[1] and U.S. EPA.[5]

Principles of the Procedure

The nitrogen, vitamin, and amino acids sources are provided by Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Beef Extract. Lactose is the fermentable cabohydrate. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capacity. Sodium Chloride maintains the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent, inhibiting many organisms except coliforms. Bromcresol Purple is used as an indicator dye; lactose-fermenting organisms turn the medium from purple to yellow with or without gas production.

Formula / Liter

Beef Extract	3 g
Enzymatic Digest of Gelatin	5 g
Lactose	7.46 g
Enzymatic Digest of Casein	9.83 g
Dipotassium Phosphate	1.35 g
Monopotassium Phosphate	1.35 g
Sodium Chloride	2.46 g
Sodium Lauryl Sulfate	0.05 g
Bromcresol Purple	0.0085 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Prepare triple strength concentration by adding 91.5 g of the medium in one liter of purified water.
2. Mix with frequent agitation to completely dissolve the medium and dispense 50 mL into a 250 mL screw-cap milk dilution bottle.
3. Autoclave at 121°C for 12 minutes. 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 and red-purple to purple.

Expected Cultural Response: Cultural response in Presence-Absence Broth at 35°C after 18-48 hour incubation.

Microorganism	Response	Reactions (Color Change – Purple to Yellow)
Enterococcus faecalis ATCC® 29212	growth	negative
Escherichia coli ATCC® 25922	growth	positive
Escherichia coli ATCC® 11775	growth	positive
Pseudomonas aeruginosa ATCC® 27853	growth	negative

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 P-A 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.[1] Report results as positive or negative for coliforms per 100 mL of sample.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. 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.

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 when stored as directed.

Limitations of the Procedure

1. The P-A test is only a presumptive test for coliforms.
2. Confirmation and differentiation of coliforms detected by the P-A test may be achieved through biochemical testing, incubation time, and temperatures as outlined in appropriate references.[1,5]
3. Extending 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.

Packaging

Presence-Absence Broth	**Code No.**	**7500A**	**500 g**
		7500B	**2 kg**
		7500C	**10 kg**

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Clark, J. A., and J. E. Pagel.** 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Microbiol. **23:**465-470.
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.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.