Purple Broth Base

M284

Purple Broth Base is recommended for the preparation of carbohydrate media used in fermentation studies for the cultural identification of pure cultures of enteric and other microorganisms.

Composition**

Ingredients	Gms / Litre
Peptone, special	10.000
Sodium chloride	5.000
Bromo cresol purple	0.020
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.02 grams in 1000 ml distilled water. Add 5 - 10 grams of the carbohydrate to be tested. Heat if necessary to dissolve the medium completely. Dispense in tubes, containing inverted Durhams tubes as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Alternatively sterilize the basal medium prepared using 900 ml distilled water and add 100 ml separately sterilized 5 - 10% solution of the desired carbohydrate to it.

Principle And Interpretation

The carbohydrates they utilize and the types and quantities of acid they produce differentiate bacteria. These differences in enzymatic activity serve as one of the important characteristics in differentiation of bacterial species. The principle of carbohydrate fermentation states that the action of organism on a carbohydrate substrate results in acidification of the medium, detected by a pH indicator dye (1).

Purple Broth Base is used for studying carbohydrate fermentation reactions, particularly in the identification of gram-negative enteric bacteria on addition of the desired carbohydrate (2, 3). Purple media were originally formulated by Vera (4). This medium is recommended by FDA (5) for fermentation studies of sugars.

Peptone special supply the essential nutrients especially nitrogen sources to the growing organisms. Sodium chloride maintains the osmotic balance of the medium. Bromocresol purple is the pH indicator, which turns yellow at acidic pH. Gas production is evident by its collection in Durham's tube. The acid produced during the fermentation of carbohydrate causes bromocresol purple, the pH indicator to turn yellow. If the carbohydrate is not utilized or fermented, the color of the medium remains unchanged or becomes more alkaline (darker purple) due to decarboxylation of the amino acids present in the medium.

The broth is inoculated with 18 to 24 hours old pure culture and incubated at 35 ± 2°C for 24 to 72 hours (upto 30 days if necessary) either in an aerobic or anaerobic atmosphere depending on the organism being tested. It is recommended (6) to add carbohydrate in 1% concentration to avoid possible reversion reactions except glucose (dextrose). If the medium containing carbohydrate is sterilized by autoclaving, precautions should be taken to use minimum amount of heat required for sterilization to avoid hydrolysis of the carbohydrate.

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear solution in tubes

Reaction
Reaction of 1.5% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with and without addition of 1% Dextrose

Cultural Response

Organism	Inoculum (CFU)	Growth	Acid (without carbohydrate)	Gas (without carbohydrate)	Acid (with1% dextrose)	Gas (with1% dextrose)
Cultural Response						
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour	positive reaction
Listeria monocytogenes ATCC 19112	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour (fermentative metabolism)	negative reaction
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	negative reaction
Staphylococcus aureus ATCC 25923	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour	negative reaction

Storage and Shelf Life

Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company
2. Ewing W. H., 1986, Edwards and Ewings identification of Enterobacteriaceae , 4th ed. Elsevier Science Publishing Co, Inc., New York, N.Y.
3. Forbes B. A., Sahm A. S., and Weissfeld D. F., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc., St. Louis, Mo.
4. Vera H. D., 1950, Am. J. Public Health, 40:1267.
5. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
6. International Organization for Standardization (ISO), 1995, Draft ISO/DIS 13720.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. Wilkins, Baltimore and I Williams.

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