



Phenylalanine Malonate Broth (Shaw and Clarke Medium)

M781

Phenylalanine Malonate Broth is used for the differentiation of members of *Enterobacteriaceae* on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine.

Composition**

Ingredients	Gms / Litre
Yeast extract	1.000
Sodium malonate	3.000
DL-Phenylalanine	2.000
Ammonium sulphate	2.000
Dipotassium phosphate	0.600
Monopotassium phosphate	0.400
Sodium chloride	2.000
Bromo thymol blue	0.025
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.03 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 115°C for 10 minutes.

Principle And Interpretation

The term enteric bacteria (or “enterics”) is generally used in reference to organisms of the Family *Enterobacteriaceae* , many members of which occur in the enteric tract of humans and animals. Members of *Enterobacteriaceae* are the most frequently encountered bacterial isolates recovered from clinical specimens. Definitive identification of the members of the *Enterobacteriaceae* may require a battery of biochemical tests (1). This medium is prepared according to the formulation developed by Shaw and Clarke (2) for differentiating gram-negative enteric bacteria on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine (4).

Yeast extract in the medium supplies nutrients to the organisms while phosphates buffer the medium. Bromothymol blue is the pH indicator. Sodium chloride maintains osmotic balance. Organisms like *Klebsiella* and *Salmonella arizonae* , which are capable of, utilizing malonate, produce an alkaline reaction and thus change the colour of the medium from light green to dark blue due to the pH indicator bromothymol blue. The colour of the medium remains light green if the organisms are malonate negative. Members of the group *Proteus* and *Providencia* are capable of deaminating phenylalanine to pyruvic acid. This reaction can be determined by the addition of few drops of 10% ferric chloride dissolved in acidified distilled water to a freshly grown culture. Deep green colour is formed due to production of pyruvic acid from phenylalanine. Malonate utilization results should be read before adding ferric chloride solution to the test tube, to detect phenylalanine deamination (3).

Quality Control

Appearance

Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellowish green coloured clear solution without any precipitate

Reaction

Reaction of 1.1% w/v aqueous solution at 25°C. pH : 6.3±0.2

pH

6.10-6.50

Cultural Response

M781: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Malonate	Phenylalanine
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	negative reaction	negative reaction
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	positive reaction, dark blue colour	negative reaction
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	negative reaction	positive reaction, green colouration after addition of 10% ferric chloride
<i>Providencia alcalifaciens</i> ATCC 9886	50-100	luxuriant	negative reaction	positive reaction, green colouration after addition of 10% ferric chloride
<i>Salmonella Arizonae</i> ATCC 13314	50-100	luxuriant	positive reaction, dark blue colour	negative reaction
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	negative reaction	negative reaction

Storage and Shelf Life

Store below 30°C in tightly closed container 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, 4 th Ed., J. B. Lippincott Company
2. Shaw C. and Clarke, 1955, J. Gen. Microbiol., 13:155.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
4. Collee J.G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone

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