Malonate Broth, Ewing Modified

M779

Malonate Broth is recommended for the differentiation of members of *Enterobacteriaceae* on the basis of malonate utilization.

Composition**

Ingredients	Gms / Litre
Yeast extract	1.000
Ammonium sulphate	2.000
Dipotassium phosphate	0.600
Monopotassium phosphate	0.400
Sodium chloride	2.000
Sodium malonate	3.000
Dextrose	0.250
Bromothymol blue	0.025
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Dissolve 9.28 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid the addition of carbon and nitrogen from other sources.

Principle And Interpretation

Leifson developed a synthetic liquid medium, which differentiated *Aerobacter* (now *Enterobacter*) from *Escherichia* species based on their ability to utilize malonate (1) where *Enterobacter* utilizes malonate and *Escherichia* does not. Ewing et al further modified this medium by the incorporation of yeast extract and dextrose (2). The addition of yeast extract, a source of vitamins, and a relatively small amount of dextrose, a minimal carbon source, is included in Ewings modification to stimulate the growth of some organisms. The medium, therefore, will support the growth of organisms that cannot utilize malonate or ammonium salt. Any spontaneous alkalinization produced by such organisms is buffered by the phosphate system and counteracted by the acid produced by the fermentation of the small amount of dextrose (3). An alkaline reaction (blue color) is produced in this medium by organisms capable of utilizing malonate and ammonium sulfate.

An organism that can simultaneously utilize sodium malonate as its carbon source and ammonium sulfate as its nitrogen source produces alkalinity due to the formation of sodium hydroxide (3). The alkali changes the color of the bromothymol blue indicator in the medium to light blue and finally to prussian blue. The color of the medium remains unchanged in the presence of an organism that cannot utilize these substances. Some malonate-negative strains produce a yellow color due to the fermentation of dextrose only, which results in increased acidity causing the pH indicator to change to yellow at a pH of 6.0. Also some malonate-positive organisms produce only a slight alkalinity that causes the results to be difficult to interpret. Therefore these tubes should be compared with an un-inoculated malonate tube (3).

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium
Bluish green coloured clear solution without any precipitate

Reaction
Reaction of 0.93% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response

M779: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours .

Organism	Inoculum (CFU)	Growth	Malonate Utilization.
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction, dark blue colour
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	positive reaction, dark blue colour
Salmonella Arizonae ATCC 13314	50-100	luxuriant	positive reaction, dark blue colour
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	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. Leifson, 1933, J. Bact., 25:329.
2. Ewing W., Davis B. and Reavis R., 1957, Public Hlth. Lab., 15:153.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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