



Bromothymol Lactose Blue Agar

M1822

Selective medium used for the isolation of gram negative bacteria from urine and faeces.

Composition**

Ingredients	Gms / Litre
Meat extract	3.000
Fish peptone	3.000
Peptone	20.000
Sodium chloride	7.500
Sodium thiosulphate	1.000
Sodium lauryl sulphate	0.150
Lactose	19.000
Bromo thymol blue	0.083
Agar	19.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 73.73 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Reactions with lactose are of great practical importance for the primary isolation of *Enterobacteria* from clinical specimens. The specimens e.g. faeces is usually plated on a lactose-containing medium on which lactose fermenters and lactose non fermenters form coloured and pale colonies respectively due to the dye incorporated. This procedure makes an immediate presumptive distinction between colonies of the true intestinal pathogens possible. *Salmonella* and *Shigella*, do not ferment lactose while the common intestinal commensals, *Escherichia* and *Klebsiella*, which do ferment lactose (1). Bromothymol Lactose Blue Agar is used for differentiating lactose fermenting and non-fermenting bacteria belonging to the family *Enterobacteriaceae*.

Meat extract, fish peptone and peptone provide essential nutrients for bacterial metabolism. Lactose provides a fermentable carbohydrate source for the enteric bacteria. Bromo thymol blue is the pH indicator for indicating acid production due to carbohydrate fermentation. The dye turns yellow at acidic pH and imparts yellow colour to the colony. Alkalinization produces a blue coloration. Sodium Lauryl sulphate inhibits gram positive organisms. Sodium chloride maintains osmotic balance.

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.9% Agar gel

Colour and Clarity of prepared medium

Greenish blue coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 7.27% w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
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Cultural Response

<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	$\geq 50\%$	yellow
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0%	-
<i>Salmonella Typhi</i> ATCC 6539	50-100	good-luxuriant	$\geq 50\%$	blue/colourless
<i>Enterococcus faecalis</i> ATCC 29212	$\geq 10^3$	inhibited	0%	-

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. Cruikshank R., Duguid J. P., Marmion B. P., Swain R. H. A., (Eds.), 1975, Medical Microbiology, The Practice of Medical Microbiology, 12th Edition, Vol. II, Churchill Livingstone

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