

Technical Data

Bromothymol Lactose Blue Agar

Intended Use:

Recommended as a selective medium for isolation of Gram-negative bacteria from urine and faeces.

Composition**	
Ingredients	g / L
HM extract#	3.000
Fish peptone	3.000
Peptone	20.000
Sodium chloride	7.500
Sodium thiosulphate	1.000
Sodium lauryl sulphate (SLS)	0.150
Lactose	19.000
Bromo thymol blue	0.083
Agar	19.000
Final pH (at 25°C)	$7.4{\pm}0.2$
**Formula adjusted, standardized to suit performance parameters	
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Equivalent to Meat extract

Directions

Suspend 73.73 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. 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*.

HM extract, fish peptone and peptone provides carbon, nitrogen compounds, long chain amino acids, vitamins and other 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.

Type of specimen

Clinical samples - Faeces, urine.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period recommended temperature.

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.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	yellow
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10 ⁴	inhibited	0%	-
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%	blue/colourless
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 ⁴	inhibited	0%	-

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

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

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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