

Technical Information

Bromothymol Lactose Blue Agar

Product Code: DM 2822

Application: - Selective medium recommended 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

Principle & 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 recommended for differentiating lactose fermenting and non-fermenting bacteria belonging to the family *Enterobacteriaceae*.

Meat extract, fish peptone and peptone supply essential nutrients for bacterial metabolism. Lactose acts as a fermentable carbohydrate and also a 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.

Methodology

Suspend 73.73 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & 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.

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.9% Agar gel

Colour and Clarity

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

pH Range

7.2 - 7.6

Cultural Response

DM 2822: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Cultural Response

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|--|-------------------|----------------|----------|------------------|
| Cultural Response <i>Escherichia coli</i> ATCC 25922 | 50-100 | good-luxuriant | >=50% | yellow |
| <i>Staphylococcus aureus</i> ATCC 25923 | >=10 ³ | inhibited | 0% | - |
| <i>Salmonella Typhi</i> ATCC 6539 | 50-100 | good-luxuriant | >=50% | blue/colourless |
| <i>Enterococcus faecalis</i> ATCC 29212 | >=10 ³ | inhibited | 0% | - |

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.**Prepared Media:** 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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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