Medium 6. Crystal Violet, Neutral Red, Bile Agar with Dextrose

MM1684

Crystal Violet, Neutral Red, Bile Agar with Dextrose is recommended for detection and enumeration of *Enterobacteria* in accordance with Indian Pharmacopoeia 2007 .

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	7.000
Yeast extract	3.000
Lactose monohydrate	10.000
Bile salts	1.500
Dextrose monohydrate	10.000
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.12 grams of dehydrated medium 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. Mix well and pour into sterile Petri plates..

Principle And Interpretation

It is selective medium recommended for detection of *Enterobacteriaceae* species as recommended by Indian Pharmacopoeia (1). Mossel et al (2,3,4) added dextrose to the medium observing improved detection of coliforms. Incubation can be carried out at different temperatures and incubation time depending upon the group of *Enterobacteriaceae* to be recovered (5).

Pancreatic digest of gelatin and yeast extract provide nitrogenous compounds and other nutrients essential for bacterial metabolism. This media is selective due to presence of the inhibitors; bile salts and crystal violet. Crystal violet inhibits gram-positive organisms especially Staphylococci. Neutral red indicator helps to detect lactose monohydrate and dextrose monohydrate fermentation. Lactose and glucose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. Sodium chloride maintains the osmotic equilibrium in the medium. The red colour is due to absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

pH
7.10-7.50

Growth Promotion Test
Growth Promotion is carried out in accordance with Indian Pharmacopoeia and cultural characteristics are observed after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony

Cultural Response

Escherichia coli ATCC 8739	50 -100	good-luxuriant	25 -100	>=50 %	pink-red
Escherichia coli NCTC 9002	50 -100	good-luxuriant	25 -100	>=50 %	pink-red
Pseudomonas aeruginosa ATCC 9027	50 -100	good-luxuriant	25 -100	>=50 %	light pink
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0 %	
Escherichia coli ATCC 25922	50 -100	luxuriant	25 -100	>=50 %	pink-red
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	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.Indian Pharmacopoeia, 2007, Govt. of India, the controller of Publication, Delhi, India.
2.Mossel D.A.A., Mengerink W.H.J. & Scholts H.H., 1962, J. Bacteriol, 84 : 381.
3.Mossel D.A.A. et al, 1978, Lab. practice, 27 No. 12 : 1049
4.Mossel D.A.A. et al, 1979, Food Protect., 42 : 470.
5.Mossel D.A.A. et al, 1986, J. Appl. Bact., 60 : 289

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