Agar Medium F (Crystal Violet, Neutral Red, Bile Agar with Glucose)

MAP1684

(ME1684/M1684B)

Intended Use:

Recommended for detection and enumeration of *Enterobacteriaceae* in accordance with EP/BP/IP.

Composition**

Ingredients	g / L
Gelatin peptone #	7.000
Yeast extract	3.000
Lactose monohydrate	10.000
Bile salts	1.500
Dextrose monohydrate (Glucose monohydrate)	10.000
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

(10.00 grams of Lactose monohydrate is equivalent to 9.50 grams of Lactose anhydrous)

(10.00 grams of Dextrose monohydrate is equivalent to 9.09 grams of Dextrose anhydrous)

Directions

Suspend 50.12 grams (the equivalent weight of dehydrated medium per litre) 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

It is selective medium recommended for detection of *Enterobacteriaceae* species as recommended by EP/BP (1,2). Mossel et al (3-5) 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 (6). Gelatin peptone 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.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical tests must be performed for confirmation.

Performance and Evaluation

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

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

7.20-7.60

Growth Promotion Test

Growth Promotion is carried out in accordance with EP/BP and cultural characteristics are observed after an incubation at 35-37°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Cultural response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony
Escherichia coli ATCC 8739 (00012*)	50 -100	good-luxuriant	25 -100	>=50 %	pink-red
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	good-luxuriant	25 -100	>=50 %	light pink
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	good-luxuriant	25 -100	>=50 %	light pink
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^3	inhibited	0	0 %	
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	25 -100	>=50 %	pink-red
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^3	inhibited	0	0 %	
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	Light pink
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	25 -100	>=50 %	pink-red

Key : *Corresponding WDCM numbers,
^ Formerly known as *Pseudomonas aeruginosa* (#) Formerly known as *Enterobacter aerogenes*

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1.European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
2.The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
3.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.

4.Mossel D.A.A. et al, 1978, Lab. practice, 27 No. 12 : 1049
5.Mossel D.A.A. et al, 1979, Food Protect., 42 : 470.
6.Mossel D.A.A. et al, 1986, J. Appl. Bact., 60 : 289
7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8.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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