



## Agar medium F(Crystal Violet, Neutral Red, Bile Agar with Glucose) ME1684

Agar Medium F is recommended for detection and enumeration of *Enterobacteria* in accordance with European Pharmacopoeia .

### Composition\*\*

| Ingredients                  | Gms / Litre |
|------------------------------|-------------|
| Pancreatic digest of gelatin | 7.000       |
| Yeast extract                | 3.000       |
| Lactose monohydrate          | 10.000      |
| Bile salts                   | 1.500       |
| Glucose monohydrate          | 10.000      |
| Sodium chloride              | 5.000       |
| Neutral red                  | 0.030       |
| Crystal violet               | 0.002       |
| Agar                         | 15.000      |
| pH after heating             | 7.4±0.2     |

\*\*Formula adjusted, standardized to suit performance parameters

### 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. DO NOT AUTOCLAVE. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

This medium is a selective medium and cited as Agar Medium F (Crystal violet, neutral red, bile agar with glucose). It is recommended for detection of *Enterobacteriaceae* species by European Pharmacopoeia from food, pharmaceutical and dietary supplement preparations (1). Mossel et al (2,3,4) added glucose 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 glucose 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 pinkish beige 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 was carried out in accordance with European Pharmacopoeia and cultural characteristics 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 |
|--|-------------------|----------------|--------------------------|----------|------------------|
| <b>Cultural Response</b><br><i>Enterobacter aerogenes</i> ATCC 13048 | 50 -100           | good-luxuriant | 25 -100                  | >=50 %   | pink-red         |
| <i>Escherichia coli</i> ATCC 25922                                   | 50 -100           | good-luxuriant | 25 -100                  | >=50 %   | pink-red         |
| <i>Salmonella Enteritidis</i> ATCC 13076                             | 50 -100           | good-luxuriant | 25 -100                  | >=50 %   | light pink       |
| <i>Staphylococcus aureus</i> ATCC 25923                              | >=10 <sup>3</sup> | inhibited      | 0                        | 0%       |                  |
| <i>Escherichia coli</i> ATCC 8739                                    | 50 -100           | luxuriant      | 25 -100                  | >=50 %   | pink-red         |
| <i>Staphylococcus aureus</i> ATCC 6538                               | >=10 <sup>3</sup> | inhibited      | 0                        | 0%       |                  |

**Storage and Shelf Life**

Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.

**Reference**

1. The European Pharmacopoeia, 2008, European Department, for the Quality of Medicines
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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