Phenylethyl Blood Agar Base (Anaerobic)

M540

Phenylethyl Blood Agar Base (Anaerobic) is used for cultivation of fastidious anaerobic bacteria.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Sodium chloride	5.000
Phenylethyl alcohol	2.500
L-Cystine	0.400
Vitamin K1	0.010
Hemin	0.005
Agar	20.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 52.92 grams in 950 ml 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. Aseptically add sterile 50 ml defibrinated sheep or rabbit blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Phenylethyl alcohol is a chemical agent that exhibits inhibitory action against gram-negative and certain gram-positive bacteria Phenylethyl Blood Agar Base (Anaerobic) is used for the isolation of obligate anaerobic gram-positive and gram-negative bacteria (2). Supplementation of medium with L-cystine permits growth of certain thiol-dependent or sulphur containing amino acids- requiring bacteria (2, 3) and fastidious Streptococci. This medium inhibits facultative anaerobic gram-negative bacteria such as *E. coli* and *Proteus* species.

Casein enzymic hydrolysate and papaic digest of soyabean meal provide nitrogen, carbon, sulfur and trace elements to the growing organisms. Addition of sheep blood provides many growth factors. Sodium chloride maintains osmotic equilibrium. Addition of phenylethanol to a nutritive medium permits the growth of gram-positive organisms but inhibits the gram-negative organisms found in the same specimen (1). Phenylethyl alcohol exerts inhibitory bacteriostatic action on gram-negative bacteria by inhibiting their DNA synthesis (4). Addition of hemin, vitamin K1 and L-cystine makes the medium more nutritious and suitable for the growth of fastidious anaerobic bacteria.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of 5%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

Reaction
Reaction of 5.29% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response

M540: Cultural characteristics observed in an anaerobic condition with added 5%w/v sterile defibrinated blood after an incubation at 35-37°C for 48-72 hours(longer if necessary).

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Bacteroides fragilis ATCC 25285	50-100	good-luxuriant	>=50%
Clostridium perfringens ATCC 13124	50-100	good-luxuriant	>=50%
Clostridium butyricum ATCC 9690	50-100	good-luxuriant	>=50%
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%
Proteus mirabilis ATCC 25933	50-100	fair-good	30-40%
Staphylococcus aureus ATCC 25923	50-100	none-poor	<=10%

Storage and Shelf Life

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

Reference

1. Lilley B. D. and Brewer J. H., 1953, J. Am. Pharm. Assoc., 42:6.
2. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
3. Allen S. D., Lombard G. L, Armfield A. Y., Thompson F. S. and Stargel M. D., Abstracts of the Annual Meeting of the American Society for Microbiology, Abstract C142, 1977, p. 59
4. Dowell, Hill and Altemeier, 1964, J. Bacteriol., 88:1811.

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