



Phenylethyl Blood Agar Base (Anaerobic)

M540

Intended Use:

Used for cultivation of fastidious anaerobic bacteria.

Composition**

Ingredients	g / L
Tryptone	15.000
Soya peptone	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 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. 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 (1). Supplementation of medium with L-cystine permits growth of certain thiol-dependent or sulphur containing amino acids- requiring bacteria (1,2) and fastidious *Streptococci*. This medium inhibits facultative anaerobic gram-negative bacteria such as *E. coli* and *Proteus* species.

Tryptone and Soya peptone 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 (3). 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.

Type of specimen

Clinical samples - faeces, pus, etc.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the pack. 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 clinical 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.

Performance and Evaluation

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

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

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
<i>Bacteroides fragilis</i> ATCC 25285	50-100	good-luxuriant	≥50%
<i>Clostridium perfringens</i> ATCC 13124 (00007*)	50-100	good-luxuriant	≥50%
<i>Clostridium butyricum</i> ATCC 9690	50-100	good-luxuriant	≥50%
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	≥50%
<i>Proteus mirabilis</i> ATCC 25933	50-100	none-poor	0 -10 %
<i>Staphylococcus aureus subsp. aureus</i> ATCC 25923 (00034*)	50-100	fair-good	30-40%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°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 (5,6).

Reference

1. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
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6. 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.

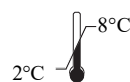
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