70133 Blood Agar, Base (Chocolate Agar, Base)

A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms like *Neisseria*, Streptococci etc. The medium is often used to observate the forms of haemolysis from pathogenic microorganisms.
This culture medium can be used without blood for setting up blood cultures and as a base for preparing special culture media.

Composition:

Ingredients	Grams/Litre
Meat extract	10.0
Peptone	10.0
Sodium chloride	5.0
Agar	15.0
Final pH 7.3+/-0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Suspend 40 g in 1 litre of distilled water. Bring to a boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. For blood agar, cool to 45-50°C and add aseptically 6% (5-10% is typically) of sterile defibrinated blood.

Principle and Interpretation:
Blood Agar Base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. For example, it was used for studying irradiated *Escherichia coli*, phages of *Clostridium perfringens*.
Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Blood Agar Base. The medium contains sodium chloride for the osmotic balance. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.
Sheep blood gives best results for Group A Streptococci. When horse blood is used, *Haemophilus haemolyticus* colonies produce haemolysis and mimic *Streptococcus*. Haemolytic patterns may vary with the source of animal blood or type of base medium used. Norton found that slight acidic pH (6.8 ± 0.2) favours distinct haemalytic reaction and is advantageous for cultivation of Streptococci and Pneumococci. The low pH helps in stabilization of red blood corpuscles and favours the formation of clear haemolysis zone.
Boiled blood agar ("chocolate agar") is prepared by heating after the blood has been added to the Blood Agar Base. Chocolate agar supplemented with 10% sterile defibrinated blood is suited for isolating *Haemophilus* and *Neisseria* species. The blood agar can be used with added phenolphthalein phosphate for the detection of phosphatase producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and carcasses and to determine salinity range of marine flavabacteria. lt was used for preparation of *Salmonella typhi* antigens.

For the selective isolation of tubercle bacilli the addition of 1 % glycerol and 25 % human blood is recommended (Tarshis and Frisch). According to Hosty et al. 0.1 % glycerol and 2.5 % human blood together with 100 IU/mol of penicillin (e.g. Cat No. PENK) is sufficient. Sondag et al. and Black et al recommend an addition of 5 mg/l gentamycine (e.g. 0.1 ml gentamycine solution Cat. No. G1397) to blood agar for the selective cultivation of *Streptococcus pneumoniae* and other Streptococci as well as bacterioides, *Clostridium* and yeasts. Mishra et al. recommend an addition of ampicillin (30mg/l Cat. No. A6140) for the selective cultivation of *Aeromonas* (ampicillin sheep blood agar or ASB agar).

Cultural characteristics after 18-48 hours at 35-37ºC.

Organisms (ATCC)	Growth	Haemolysis
Neisseria meningitidis (13090)	+++	-
Staphylococcus aureus (25923)	+++	beta
Streptococcus pyrogenes (19615)	+++	beta
Streptococcus pneumoniae (6303)	+++	alpha
Clostridium perfringens (13124)	+++	beta
Bordetella bronchiseptica (4617)	+++	-

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Precautions and Disclaimer

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