B116	**BLOOD AGAR BASE NO.2**
Formula	
Ingredients:	**gms/lit.**
Proteose peptone	15.00
Liver extract	2.50
Yeast extract	5.00
Sodium chloride	5.00
Agar	15.00

Final pH (at 25°C) : 7.4 ± 0.2

Directions :

Suspend 21.25 grams in 500 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 and aseptically add 7% v/v sterile defibrinated blood.

For Brucella species: Add rehydrated contents of 1 vial of Brucella Selective Supplement, Modified (BF012) to 500 ml sterile molten base.

For Campylobacter species: Add rehydrated contents of 1 vial of Campylobacter Supplement - I (BF013) or Campylobacter Supplement – II, Modified (BF014) or Campylobacter Supplement - III (BF015) or Campylobacter Growth Supplement (BF016) to 500 ml sterile molten base.

For Streptococcus species: Add rehydrated contents of 1 vial of Strepto Supplement (BF017) to 500 ml sterile molten base. Mix well and pour into sterile Petri plates.

Principle:

Blood Agar Base formulations have been prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms.

Proteose Peptone is the nitrogen source for Blood Agar Base No. 2 while Yeast Extract and Liver Digest provide essential carbon, vitamin, nitrogen and amino acids sources. It contains Sodium Chloride to maintain osmotic balance and Agar as a solidifying agent. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood or type of base medium used. Chocolate agar for isolating Haemophilus and Neisseria species can be prepared from blood agar base No. 2 by supplementing the medium with 10% sterile defibrinated blood.

QC Tests – (I)Dehydrated Medium		
	Colour :	Cream to yellow
	Appearance :	Homogeneous Free Flowing powder
(II)Rehydrated medium		
	pH (post autoclaving/heating) :	7.4 ± 0.2
	Colour (post autoclaving/heating) :	A) Basal medium : Light amber to yellow B) After addition of 7% sterile defibrinated blood: Cherry red.
	Clarity (post autoclaving/heating) :	A : Clear to slightly opalescent B : Opaque
(III)Q.C. Test Microbiological		

Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

MICROORGANISM (ATCC)	GROWTH WITH BLOOD	HAEMOLYSIS	GROWTH W/O BLOOD
Neisseria meningitidis (13090)	Luxuriant	none	fair
Streptococcus pneumoniae (6303)	Luxuriant	alpha	fair-good
Streptococcus pyogenes (19615)	Luxuriant	beta	fair-good
Staphylococcus aureus (25923)	Luxuriant	beta	good

Refer disclaimer Overleaf

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Precautions :	1. For Laboratory Use.				
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.				
Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
	2. Blood Agar Base media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.				
	3. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
	4. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta – hemolytic on horse, human and rabbit blood agar and alpha – hemolytic on sheep blood agar.				
	5. Colonies of Haemophilus haemolyticus are beta – hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta – hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of H. haemolyticus.				
	5. Atmosphere of incubation has been shown to influence hemolytic reactions of beta – hemolytic streptococci. For optimal performance, incubated blood agar base media under increased CO_2 or anaerobic conditions.				
Use :	It is used to permit the maximum recovery of streptococci, pneumococci and other fastidious pathogenic microorganisms without interfering with their haemolytic reactions.				
Storage :	Dehydrated medium-below 30°C Prepared medium- Between 2 to 8°C.				
Packing :	500 gm. bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
B116	42.5 g/l	11.764 L	7.4 $\pm$ 0.2	5-10% v/v sterile defibrinated blood.	121^{0}C / 15 minutes

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