Dehydrated Culture Media

BLOOD AGAR BASE

NO.2

Code: CM0271

An improved Blood Agar Base possessing enhanced nutritional properties suitable for the cultivation of fastidious pathogens and other micro-organisms.

Typical Formula*

	gm/litre
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0

pH 7.4 ± 0.2 @ 25°C

*** Adjusted as required to meet performance standards**

Directions
Suspend 40g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C and add 7% sterile blood.

Mix with gentle rotation and pour into sterile dishes or other containers.

Description
Oxoid Blood Agar Base No.2 was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of delicate organisms without interfering with their haemolytic reactions. In comparison with fresh digest agar, Blood Agar Base No.2 may be shown to have equal or superior growth promoting properties and chromogenic bacteria grown on the Oxoid medium show enhanced pigment formation. Comparison with many other blood agars has shown that with Oxoid Blood Agar Base No.2 growth of many bacteria - especially the fastidious *streptococci* and *pneumococci* - is considerably improved, as shown by luxuriant and early colonial development.

Oxoid Blood Agar Base No.2 is specified by the American Food and Drug Administration for the preparation of sheep blood agar[1].

Phillips[2], described an improved medium for sporulation of *Clostridium perfringens* based on Blood Agar Base No.2 to which are added lysed horse blood, bile, sodium bicarbonate and quinoline.

The medium induced significant sporulation in all of 100 strains of *Clostridium perfringens* isolated from human faeces.

Brucella:
To prepare a selective medium add Brucella Selective Supplement SR0083 or Modified Brucella Selective Supplement SR0209 to 500ml of sterile, molten Blood Agar Base No.2 containing 5-10% v/v inactivated horse serum and 1% w/v dextrose[2,3].

Campylobacter:
To prepare a selective medium add Campylobacter Supplement (Skirrow)[5] SR0069 or Campylobacter Supplement (Butzler)[6] SR0085 or Campylobacter Supplement (Blaser-Wang)[7] to 500ml of sterile, molten Blood Agar Base No.2 containing Campylobacter Growth Supplement SR0232 as required and 5-7% v/v horse or sheep blood (SR0048, SR0050 or SR0051).

Haemophilus:
For the primary isolation of *Haemophilus* species from specimens containing a mixed flora, use Blood Agar Base No.2 with added Defibrinated Horse Blood SR0050. Even better results may be obtained using the horse blood agar plates with half of each spread with 2 drops of 10% saponin[9]. Where haemolytic reactions are not important, for example when dealing with pure cultures, the Base may be used to prepare chocolate agar. Add 10% of Defibrinated Horse Blood code SR0050 to the Base at 80°C and maintain at this temperature for 5 to 10 minutes, agitating frequently. Cool to 50°C, mix well and pour plates.

Roberts, Higgs and Cole used Blood Agar Base No.2 as the basis of a medium which is selective for *Haemophilus* spp. in primary culture of clinical specimens. The medium distinguishes *Haemophilus*

influenzae and Haemophilus parainfluenzae by differences in colony colour[10].
A selective chocolate blood agar for the culture of *Haemophilus influenzae* from respiratory secretions of cystic fibrosis patients has been described[11]. The medium is based on Blood Agar Base No. 2 to which 7% v/v horse blood and 8 mg/litre of cefsulodin is added. Growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* on this medium is inhibited.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Straw coloured gel

Quality control

Positive controls:
Expected results
Blood Agar
Staphylococcus aureus ATCC® 25923 *
Good growth; white/grey colonies
Streptococcus pyogenes ATCC® 19615 *
Good growth; pale straw coloured colonies; ß-heamolysis
Haemophilus influenzae ATCC® 35056 Good growth; colourless colonies
Brucella Agar
Brucella abortus ATCC® 4315 Good growth
Campylobacter Agar
Campylobacter jejuni ATCC® 29428 * Good growth; grey/brown colonies
Negative controls:
Blood Agar
Uninoculated medium No change
Brucella Agar & Campylobacter Agar
Escherichia coli ATCC® 25922 * Inhibited

*** This organism is available as a Culti-Loop®**

Precautions
Brucella cultures are highly infective and must be handled under properly protected conditions.
Incubate in 5-10% carbon dioxide atmosphere for 24-48 hours.

References
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