Columbia Blood Agar Base

**Intended Use:**
Recommended for preparation of blood agar, chocolate agar and for preparation of various selective and identification media and isolation of organisms from clinical and non clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
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</thead>
<tbody>
<tr>
<td>Peptone, special</td>
<td>23.000</td>
</tr>
<tr>
<td>Corn starch</td>
<td>1.000</td>
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<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>7.3±0.2</td>
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</table>

**Directions**

Suspend 44.0 grams of in 1000 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 before adding heat sensitive compounds.

For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.

For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.

The medium can be made selective by adding different antimicrobials to sterile base.

For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (FD006) or Campylobacter Supplement- II, (Butzler) (FD007) or Campylobacter Supplement- III (Skirrow) (FD008) or Campylobacter Selective Supplement (FD090) or Campylobacter Supplement- VI (Butzler) (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.

For *Gardnerella* species: Add rehydrated contents of 1 vial of G.Vaginalis Selective Supplement (FD056) to 500 ml sterile molten base.

For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (FD030) or Strepto Supplement (FD031) or Streptococcus Selective Supplement (FD119) to 500 ml sterile molten base.

**Principle And Interpretation**

Columbia Blood Agar Base was devised by Ellner et al (4). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (5,6). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (3). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.
Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae*, lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO$_2$.

Precaution: *Brucella* cultures are highly infective and must be handled carefully; incubate in 5-10% CO$_2$. *Campylobacter* species are best grown at 42°C in a micro aerophillic atmosphere. Plates with *Gardenerella* supplements plates should be incubated at 35°C for 48 hours containing 7% CO$_2$.

**Type of specimen**
Clinical samples : blood, respiratory exudates.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
In Vitro diagnostic use only. Read the label before opening the container. 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. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. As this medium have a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction which may be mistaken for the alpha haemolysis.
3. Carry out confirmatory tests of all the colonies.

**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 1.5% 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 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

**pH**
7.10-7.50

**Growth Promotion Test**
In accordance with the harmonized method of USP/EP/BP/JP.

**Cultural Response**
Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 24-48 hours.
Organism | Inoculum (CFU) | Growth | Recovery | Haemolysis |
---|---|---|---|---|
*Neisseria meningitidis* ATCC 13090 | 50-100 | luxuriant | >=70% | none |
*Staphylococcus aureus* subsp. aureus ATCC 25923 (00034*) | 50-100 | luxuriant | >=70% | beta / gamma |
*Staphylococcus epidermidis* ATCC 12228 (00036*) | 50-100 | luxuriant | >=70% | gamma |
*Staphylococcus aureus* subsp. aureus ATCC 6538 (00032*) | 50-100 | luxuriant | >=70% | beta / gamma |
*Streptococcus pneumoniae* ATCC 6303 | 50-100 | luxuriant | >=70% | alpha |
*Streptococcus pyogenes* ATCC 19615 | 50-100 | luxuriant | >=70% | beta |
*Clostridium sporogenes* ATCC 19404 (00008*) | 50-100 | luxuriant | >=50 % | |
*Clostridium sporogenes* ATCC 11437 | 50-100 | good-luxuriant | >=50 % | |
*Clostridium perfringens* ATCC 13124 (00007*) | 50-100 | luxuriant | >=50 % | |
*Clostridium perfringens* ATCC 12934 | 50-100 | luxuriant | >=50 % | |

Key : *Corresponding WDCM numbers.

**Storage and Shelf Life**
Store between 10-30°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. Use before expiry date on the label. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

**Reference**
In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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