**Columbia Broth Base**

**Intended Use:**
Recommended used as a general purpose medium and also for the cultivation of fastidious organisms from clinical specimens.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone special</td>
<td>10.000</td>
</tr>
<tr>
<td>Biopeptone</td>
<td>10.000</td>
</tr>
<tr>
<td>HI powder</td>
<td>3.000</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride</td>
<td>0.100</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>2.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.100</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.020</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>0.600</td>
</tr>
<tr>
<td>Tris (hydroxymethyl) aminomethane</td>
<td>0.830</td>
</tr>
<tr>
<td>Tris (hydroxymethyl) aminomethane hydrochloride</td>
<td>2.860</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.5±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 35.01 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. If desired, SPS (Sodium polyanethol sulphonate) may be added in a final concentration of 0.01%. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

**Principle And Interpretation**

Morello and Ellner in 1969 devised a liquid medium for the recovery of microorganisms from blood cultures (4). This medium was devised from Columbia Blood Agar Base previously formulated by Ellner et al (1). While studying they found that Columbia Broth was superior to a commonly used general-purpose broth for faster growth of *Staphylococcus aureus*, *Escherichia coli*, viridans Streptococci and *Enterococcus* groups. In the formulation the increased concentration of cystine is provided for improved recovery of both aerobic and anaerobic microorganisms from blood specimens. Columbia Broth Base supplemented with SPS (Sodium Polyanethol Sulphonate), a polyanionic anticoagulant inhibits complement and lysozyme activity, interferes with phagocytosis and inactivates aminoglycosides (5). The presence of CO₂ is stimulatory for many organisms. It is an excellent blood culture medium (2). It differs from the agar base in that the cornstarch is omitted to reduce opalescence (4) and salts have been included.

Medium contains peptone special, biopeptone and HI powder to support luxurious growth of the organisms. Dextrose is added as a carbon and energy source. The medium is buffered with tris buffer. The addition of salts was found to be beneficial for the recovery of organisms. L-Cysteine HCL is the reducing agent. Magnesium & iron are added to facilitate organism growth.

Tube media should be inoculated with 1 to 2 drops of the liquid specimen using a sterile pipette. Swab specimens may be inserted into the broth after inoculation of the plated media. Liquid media should be reduced by placing the tubes with caps loosened under anaerobic conditions for 18-24 hours prior to inoculation for anaerobic incubation. Alternatively, it can be reduced immediately before use by boiling with caps loosened and cooling to room temperature with tightened caps, before inoculation. Growth in tubes is indicated by presence of turbidity compared to an uninoculated control. If growth appears, cultures should be subcultured onto appropriate media. Addition of SPS is inhibitory to *Neisseria* species, and thus 1.2% gelatin addition may counteract the inhibitory effect.

**Type of specimen**

Clinical samples - Blood

---

Please refer disclaimer Overleaf.
Specimen Collection and Handling:
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
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. 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.
3. Further biochemical and serological tests must be carried out for further identification.

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

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent solution, may have a fine precipitate.

Reaction
Reaction of 3.5% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours

Organism | Inoculum (CFU) | Growth
---|---|---
Clostridium perfringens ATCC 12924 | 50-100 | good-luxuriant
Neisseria meningitidis ATCC 13090 | 50-100 | good-luxuriant
Staphylococcus aureus subsp. aureus ATCC 25923 (08034*) | 50-100 | good-luxuriant
Streptococcus mitis ATCC 9811 | 50-100 | good-luxuriant
Streptococcus pyogenes ATCC 19615 | 50-100 | good-luxuriant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Please refer disclaimer Overleaf.
Reference