Chocolate Agar Base

Intended use
Recommended for the isolation of *Neisseria gonorrhoeae* from chronic and acute cases of gonococcal infections.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.3±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

**Directions**

Suspend 45.5 grams in 495 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. Aseptically add equal amount (495 ml) of sterile 2% Haemoglobin solution (FD022). Also add the contents or one vial of Yeast Autolysate Supplement (FD027) or Vitamino Growth Supplement (FD025) reconstituted as directed. Mix well before pouring. When single strength medium is desired, suspend 45.5 grams in 1000 ml distilled water.

**Principle And Interpretation**

*Neisseria gonorrhoeae* is a gram-negative bacteria and the causative agent of gonorrhea, however it is also occasionally found in the throat. The cultivation medium for gonococci should ideally be a rich nutrients base with blood, either partially lysed or completely lysed. The diagnosis and control of gonorrhea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoea*.

Chocolate Agar Base, with the addition of supplements, gives excellent growth of the gonococcus without overgrowth by contaminating organisms. G.C. Agar (M434) can also be used in place of Chocolate Agar Base, which gives slightly better results than Chocolate Agar (4). The diagnosis and control of gonorrhea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoea*.

Interest in the cultural procedure for the diagnosis of gonococcal infection was stimulated by Ruys and Jens (9), Mcleod and co-workers (8), Thompson (7), Leahy and Carpenter (1), Carpenter, Leahy and Wilson (2) and Carpenter (10), who clearly demonstrated the superiority of this method over the microscopic technique. Chocolate Agar Base with addition of supplement not only supports the growth of the gonococcus in pure culture but also permits its development from the mixed flora encountered in chronic gonococcal infections. Carpenter (3) reported that this medium and Haemoglobin (FD022) is useful for cultural detection of the gonococcus.

**Type of specimen**
Clinical samples - Blood, vaginal samples.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Please refer disclaimer Overleaf.
**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. Further biochemical tests must be carried out for confirmation.

**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 haemoglobin : Chocolate brown coloured opaque gel forms in Petri plates.

**Reaction**
Reaction of 4.5% w/v aqueous solution at 25°C. pH : 7.3±0.2

**pH**
7.10-7.50

**Cultural Response**
Cultural characteristics observed with added 2% haemoglobin solution (FD022), Yeast autolysate Supplement (FD027) or Vitamino Growth Supplement (FD025), after an incubation at 35-37°C for 40-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neisseria gonorrhoeae ATCC 19424</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Neisseria meningitidis ATCC 13090</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Streptococcus pneumoniae ATCC 6303</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Streptococcus pyogenes ATCC 19615</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Haemophilus influenzae ATCC 19418</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
</tbody>
</table>

**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. 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 (5,6).

Please refer disclaimer Overleaf.
Reference

7. J. Infectious Diseases, 61:129:1937

Disclaimer:
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.