Anaerobic Agar

Intended Use:
Recommended for the cultivation of anaerobic bacteria, especially Clostridium species and other anaerobic organisms from clinical and non-clinical samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium formaldehyde Sulfoxylate</td>
<td>1.000</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>20.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 58.0 grams 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Anaerobic Agar was originally designed for surface cultivation of members of the genus Clostridium and other anaerobic organisms on plates (2). This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology as colonies can be readily seen on the light coloured agar and are easily accessible (5,3). Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements (3). Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor (1).

This medium contains sodium thioglycollate and sodium formaldehyde sulfoxylate that provide adequate anaerobiosis which is indicated by methylene blue present in the medium which yields blue colour to medium in presence of oxygen. Tryptone and dextrose provide essential nutrients while sodium chloride maintains osmotic equilibrium.

Dispense 50-60 ml medium per 95 x 20 mm plate. For best results, use porous tops for the plates during solidification to get the dry surface. Inoculation can be done by streaking or smearing. Cover the inoculated plate with sterile Brewer Anaerobic Petri dish cover. Incubate aerobically, as desired. When standard plates are used, dispense 0.1 to 1.0 ml of inoculum into plates and mix with 20 - 25 ml of sterile medium. After solidification, incubate anaerobically as required by particular organism under study. Methylene blue is inhibitory to some anaerobic microorganisms.

Type of specimen
Clinical- stool, blood, abscess

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

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.

Please refer disclaimer Overleaf.
Limitations
1. Ensure that the clinical samples are properly transported under anaerobic conditions.
2. Proper anaerobic conditions must be maintained for optimal recovery of organisms.
3. Methylene blue is toxic to certain anaerobes.

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 2.0% agar gel.

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates that becomes greenish due to aeration on standing

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH: 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>ATCC 12924</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>ATCC 11437</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium butyricum</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>ATCC 13732</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 (3,4).

Reference
In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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Disclaimer:

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