**Clostridial Agar**

**Intended use**

Recommended for the selective isolation of pathogenic Clostridia from mixed flora.

### Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>17.000</td>
</tr>
<tr>
<td>Soya peptone</td>
<td>3.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>6.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.500</td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>1.800</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.250</td>
</tr>
<tr>
<td>Sodium formaldehyde sulphoxylate</td>
<td>1.000</td>
</tr>
<tr>
<td>Neomycin sulphate</td>
<td>0.150</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.200</td>
</tr>
<tr>
<td>Agar</td>
<td>14.500</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

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

### Directions

Suspend 46.4 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118°C for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

One of the major species of anaerobic bacteria to cause disease in humans is *Clostridium*. *Clostridium* species cause tetanus and gas gangrene that ultimately leads to tissue damage. Another *Clostridium* species produces the lethal botulinum toxin, the causative agent of botulism (1). Clostridial Agar formulated by Vera is recommended for the selective isolation of pathogenic Clostridia form mixed flora (7). The media is well supplemented to support luxuriant growth of *Clostridium* species.

Tryptone and soya peptone provide the nitrogenous and carbonaceous compounds, long chain amino acids and other essential nutrients, mainly the nitrogen compounds. Dextrose serves as the carbon or fermentable carbohydrate source. L-cystine is an amino acid, which promotes the growth of Clostridia. Sodium thioglycollate and sodium formaldehyde sulphoxylate are the reducing agents that help to create low oxidation-reduction potential enabling the growth of Clostridia. Accompanying enteric bacteria including *Proteus, Pseudomonas* and *Bacillus* species are inhibited by neomycin sulphate and sodium azide incorporated in the medium. The ideal method of inoculation of Clostridial Agar is direct inoculation of sterile, cooled medium with the specimen (in tubes). Alternatively agar plates of the medium can also be inoculated by streaking.

### Type of specimen

Clinical samples - Blood; Food and dairy samples.

### Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,6,8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions:

In Vitro diagnostic use. 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.
**Limitation**
1. Further biochemical test 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 beige homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.45% Agar gel

**Colour and Clarity of prepared medium**
Yellow coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.64% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**
6.80-7.20

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens ATCC 12924</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Clostridium sporogenes ATCC 11437</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Clostridium tetani ATCC 10779</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td>Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
</tr>
</tbody>
</table>

* - 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 (3,4).

**Reference**

Please refer disclaimer Overleaf.
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