Liver Veal Agar is recommended for the cultivation of fastidious anaerobic organisms.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver, infusion from</td>
<td>50.000</td>
</tr>
<tr>
<td>Veal, infusion from</td>
<td>500.000</td>
</tr>
<tr>
<td>Proteose peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>1.300</td>
</tr>
<tr>
<td>Peptone, special</td>
<td>1.300</td>
</tr>
<tr>
<td>Gelatin</td>
<td>20.000</td>
</tr>
<tr>
<td>Starch, soluble</td>
<td>10.000</td>
</tr>
<tr>
<td>Casein, purified</td>
<td>2.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>2.000</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 97 grams in 1000 ml warm distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Anaerobic bacteria live in an oxygen-free environment. Some anaerobic bacteria actually die if oxygen is present while others fail to grow and multiply (1). One of the methods of cultivation of anaerobes is using the Sprays medium by using the anaerobic culture dish (2). Liver Veal Agar is formulated as per the medium of Spray (3). Liver Veal Agar is recommended by APHA (4) and the FDA Bacteriological Analytical Manual (BAM) (5). Liver Veal Agar on supplementation of 50% egg yolk is recommended for the cultivation of anaerobic organisms (4-6). The medium is highly nutritious and therefore is an excellent medium for growth of sporulating anaerobic bacteria.

Both the infusions, peptones, casein enzymic hydrolysate and gelatin serve as sources of carbon, nitrogen, amino acids and various vitamins. Dextrose serves as the carbon source. Starch enhances growth of anaerobic bacteria. Spray reported isolation of *Clostridium perfringens* within 6 hours of inoculation and *Clostridium tetani* within 8 hours. When the medium is inoculated with a small inoculum, gas production is not evident. Spray recommended that the medium should be taken directly from the sterilizer or should be boiled for 10 minutes to drive off dissolved oxygen and cooled without agitation. Serial inoculations are made and the medium is poured into plates. After solidification, 5 ml sterile Liver Veal Agar is poured over the medium as a cover layer to prevent the spreading of surface colonies.

Refer standard procedures for isolation and cultivation of anaerobic bacteria (7, 8).

*C. botulinum* and *C. tetani* are highly hazardous and extreme care should be taken while handling these cultures.

**Quality Control**

**Appearance**
Light yellow to brownish yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Amber coloured clear to slightly opalescent gel forms in Petri plates, may have slight precipitate.
Reaction
Reaction of 9.7% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M176: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (under the atmospheric requirement of organism).

Organism | Growth
--- | ---
*Clostridium botulinum* ATCC 25763 | luxuriant
*Clostridium tetani* ATCC 10709 | luxuriant
*Neisseria meningitidis* ATCC 13090 | luxuriant
*Streptococcus pneumoniae* ATCC 6303 | luxuriant

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference