Gelatin Agar

Gelatin Agar is recommended for cultivation and identification of Vibrio species.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin</td>
<td>30.000</td>
</tr>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 65 grams in warn preheated 1000 ml 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**

Members of the genus Vibrio are facultative anaerobes capable of both respiratory and fermentative metabolism. The natural habitat for Vibrio species is aquatic, in both fresh water and salt water. The growth and biochemical reactivity of most species are enhanced in different test media supplemented with 1-2% sodium chloride. Vibrios are fairly easy to isolate from both clinical and environmental material, though some species may require growth factors and/or vitamins. Media can be made selective for Vibrio species by adding appropriate selective agents (1). High concentrations of NaCl and alkaline pH have also been used to select certain Vibrio species, based on the ability of most Vibrio species to grow at pH values above 8.0 and at 3% or higher concentrations of NaCl. Gelatin Agar is formulated in accordance with APHA (2) for the cultivation and characterization of Vibrio species from foods and faeces. Clinical specimens must be obtained early in the disease as possible because the duration of excretion of the pathogen is short.

Weigh 25 grams of sample such as seafood or vegetables either blended or cut into small pieces and add into 2 flasks. Add 225 ml Alkaline Peptone Water (M618) to one flask and 225 ml of Glucose Phosphate Broth (M070) in another flask. Mix well. Incubate at 35° ± 2°C for 6 to 8 hours. Inoculate one loopful from each flask on the non-selective Gelatin Agar.

*V.cholerae* appear transparent and usually have a characteristic cloudy zone around colony, which becomes more definite after few minutes of refrigeration. When these colonies are viewed in oblique light they appear iridescent green to bronze coloured and finely granular.

**Quality Control**

**Appearance**

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel and 3.0% Gelatin gel

**Colour and Clarity of prepared medium**

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 6.5% w/v aqueous solution at 25°C; pH : 7.2±0.2

**pH**

7.00-7.40

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Gelatin liquefaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vibrio cholerae ATCC 15748</strong></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>positive reaction, clear zone around the colony within 24-48 hours</td>
</tr>
<tr>
<td><strong>Vibrio parahaemolyticus ATCC 17802</strong></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>positive reaction, clear zone around the colony within 24-48 hours</td>
</tr>
</tbody>
</table>

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

**Reference**