Sheep Blood Agar Base, Modified

M1956

Used for cultivation and studying haemolytic reactions of *Bacillus cereus* in accordance with ISO 21871:2006.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of casein</td>
<td>15.000</td>
</tr>
<tr>
<td>Enzymatic digest of soya</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>12.500</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>7.3±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 37.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% w/v sterile sheep blood. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Haemolysins are exotoxins produced by bacteria that lyse red blood cells. The haemolytic reaction can be visualized on blood agar plates. On blood agar plates colonies of haemolytic bacteria may be surrounded by clear, colourless zone where the red blood cells have been lysed and the haemoglobin destroyed to a colourless compound. This is beta haemolysis. Other types of bacteria can reduce haemoglobin to methaemoglobin which produces a greenish zone around the colonies and is called alpha haemolysis (1). Gamma haemolysis is no haemolysis where no change in the medium is observed (2).

*Bacillus cereus* is Gram-positive aerobic or facultatively anaerobic, motile, spore forming, rod shaped bacterium that is widely distributed environmentally. *B. cereus* is associated mainly with food poisoning it is increasingly reported to be cause of serious and fatal non-gastrointestinal-tract infections Sheep Blood Agar Base, Modified with added sheep blood was developed to allow maximum recovery of *B. cereus* without interfering with their haemolytic reactions. This medium is formulated in accordance with ISO(3). It was formulated to be compatible with sheep blood and give improved haemolytic reactions of organisms.

Enzymatic digest of casein and soya peptone provide nitrogen, carbon, amino acids and vitamins. Sodium chloride maintains the osmotic balance.

**Quality Control**

**Appearance**
Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.25% Agar gel

**Colour and Clarity of prepared medium**
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

**Reaction**
Reaction of 3.75% w/v aqueous solution at 25°C. pH: 7.3±0.2

**pH**
7.10-7.50

**Cultural Response**
Cultural characteristics observed with added 5% w/v sterile defibrinated blood after an incubation at 35-37°C for 18-48 hours.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Inoculum (CFU)</th>
<th>Recovery</th>
<th>Haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus ATCC 10876</td>
<td>luxuriant</td>
<td>50-100</td>
<td>&gt;=70%</td>
<td>beta</td>
</tr>
</tbody>
</table>

### Storage and Shelf Life

### Reference