**Brucella Agar Base with Hemin and Vitamin K**

Brucella Agar Base with Hemin and Vitamin K is recommended for the isolation, cultivation and subculture of *Brucella* species and other anaerobes.

**Composition***

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>10.000</td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium bisulphite</td>
<td>0.100</td>
</tr>
<tr>
<td>Hemin</td>
<td>0.010</td>
</tr>
<tr>
<td>Vitamin K1</td>
<td>0.010</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)**

7.0±0.2

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

**Directions**

Suspend 43.12 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5% v/v sterile defibrinated sheep blood. Mix well before pouring into sterile Petri plates.

**Principle And Interpretation**

The agents of brucellosis, *Brucella* species are normal flora of the genital and urinary tracts of many animals including goats, pigs, cows and dogs. Most humans acquire the disease through ingestion of contaminating milk or through occupational exposure; the disease is particularly common among abattoir workers (1).

Brucella Agar Base w/ Hemin and Vitamin K1 is a modified (4, 5, 6) and highly enriched medium, which can be used for the isolation of *Brucella* and other anaerobic bacteria (2, 3).

The medium contain casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract as sources of carbon, nitrogen and essential growth nutrients including B-complex vitamins. Dextrose serves as a source of energy. Addition of blood provides nutrients and helps to differentiate hemolytic organisms (2, 3). Presence of hemin and Vitamin K1 supports growth of other fastidious bacteria like *Bacteroides* species and gram-positive spore bearers like *Clostridium* species (7). The specimen should be inoculated onto the plate (reduced earlier by placing under anaerobic conditions for 18-24 hrs) as early as possible. Swab cultures are directly streaked. Non-swab cultures are inoculated using an inoculating loop.

Incubation is carried out anaerobically at 35°C for at least 48 hours; however, negative results should be reported only after incubation for 7 days.

**Quality Control**

**Appearance**

Light yellow to tan homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% 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 4.31% w/v aqueous solution at 25°C: pH : 7.0±0.2
pH
6.80-7.20

Cultural Response
M1039: Cultural characteristics observed in presence of 10% CO2 with added 5% v/v sterile defibrinated sheep blood, after an incubation at 35-37°C for 48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis ATCC 25285</td>
<td>good-luxuriant</td>
</tr>
<tr>
<td>Clostridium perfringens ATCC 13124</td>
<td>good-luxuriant</td>
</tr>
</tbody>
</table>

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