Brucella Vitamin K1 Blood Agar Base

Brucella Vitamin K1 Blood Agar Base is recommended for the isolation, cultivation and subculture of Brucella species and other anaerobes.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</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>Dextrose</td>
<td>1.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium bisulphite</td>
<td>0.100</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 43.1 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 45-50°C and aseptically add 5% v/v sterile defibrinated sheep blood. Aseptically add sterile Vitamin K1 solution to give a final concentration of 10 mcg/ml. 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 was originally developed for isolation of Brucella species. But with the supplementation of blood and other nutritious substances, it can be used for growth and isolation of various fastidious organisms. Brucella Blood Agar Base was modified by the addition of Vitamin K1 by Sutter et al (4). Brucella Vitamin K1 Blood Agar Base is a highly enriched medium, which can be used for the isolation of anaerobic bacteria (2, 3).

The medium contains 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). Addition of hemin and Vitamin K1 supports growth of other fastidious bacteria like Bacteroides species and gram-positive spore bearers like Clostridium species (5). 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 an 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 K1 & 5% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates

**Reaction**

Reaction of 4.3% w/v aqueous solution at 25°C. pH: 7.0±0.2
pH
6.80-7.20

Cultural Response
M823: Cultural characteristics observed in presence of 10% CO₂, under anaerobic condition with added 5%v/v defibrinated sheep blood and Vitamin K1, 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

Revision : 2 / 2015

Disclaimer :
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