Brucella Agar Base, Modified

Intended Use:
Recommended for cultivation of Campylobacter species.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>15.000</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium bisulphite</td>
<td>0.100</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 22.05 grams in 500 ml purified / 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 sterile 5% v/v inactivated horse serum (RM1239, Inactivate by heating at 56°C for 30 minutes) and rehydrated contents of one vial of Campylobacter Supplement III (Skirrow)(FD008) and sterile reconstituted contents of one vial of Campylobacter Growth Supplement (FD009). Mix well before pouring into sterile petri plates.

Principle And Interpretation
This medium is formulated so as to support luxuriant growth of fastidious bacteria like Campylobacter and Brucella species (3).
Peptone, Tryptone provide organic nitrogen to the organisms. Yeast extract also supply some nitrogenous nutrients but mainly it serves as a source of Vitamin B complex. Dextrose serves as an energy source. It can be enriched with 5% v/v sterile defibrinated horse blood. For selective isolation of Brucella species, antibiotic mixtures are incorporated into the base (6,7,8). Farrel and Robinson formulated a highly selective antibiotic medium (2). Ethyl violet and Circulin, which were recommended originally, are no longer used (1). When non-selective medium is required, Brucella Broth Base may be employed with the addition of serum only (i.e. without antibiotics).
It is suggested in case of broth medium that half the tubes be incubated in the normal atmosphere, and half in a 10% CO2 enriched atmosphere. Brucella species are highly infectious and so extreme care should be taken while handling. All presumptive anaerobic organisms must be further confirmed by the tests.

Type of specimen
Clinical material: stool, body tissue or fluids

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Please refer disclaimer Overleaf.
Limitations
1. C. jejuni is thermophilic and should be incubated at 42ºC. Otherwise, growth of colonies may be delayed.
2. Campylobacter species require a microaerophilic atmosphere containing approximately 10% CO₂ for optimal recovery.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Cream to yellow coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel or solution forms in petri plates

Reaction
Reaction of 4.3% w/v aqueous solution at 25ºC. pH : 7.0±0.2

Cultural Response
Cultural characteristics observed after an incubation at 35-37ºC for 24-48 hours under 10% CO₂ with added sterile 5% v/v inactivated horse serum (RM1239).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni ATCC</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>29428 (00156*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter coli ATCC</td>
<td>50-100</td>
<td>good luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>33559</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td>25922 (00013*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus subsp.</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td>aureus ATCC 25923</td>
<td>(00034*)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30ºC in a tightly closed container and the prepared medium at 2-8ºC. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

Please refer disclaimer Overleaf.
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