Reinforced Clostridial Broth is used for the cultivation and enumeration of *Clostridia* 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>Beef extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Starch, soluble</td>
<td>1.000</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>3.000</td>
</tr>
<tr>
<td>Agar</td>
<td>0.500</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 38.00 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes. Mix well and dispense as desired.

**Principle And Interpretation**

Reinforced Clostridial Broth is formulated by Hirsch and Grinsted (1). It can be used to initiate growth from small inocula and to obtain the highest viable count of *Clostridia*. Barnes and Ingram used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens* (2). It can be used in studies of spore forming anaerobes, especially *Clostridium butyricum* in cheese, for enumeration of Clostridia in tube dilution counts or for preparation of plates for isolation (3). Other spore forming anaerobes, *Streptococci* and *Lactobacilli* also grow in this media. This is a nonselective enrichment media. Casein enzymic hydrolysate, yeast extract, beef extract, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of *Clostridia*. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. This media can be made selective by addition of 15-20 mg Polymyxin B per litre of media (1).

**Quality Control**

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

**Colour and Clarity of prepared medium**
Light yellow coloured clear solution in tubes.

**Reaction**
Reaction of 3.8% w/v aqueous solution at 25°C. pH: 6.8±0.2

**pH**
6.60-7.00

**Cultural Response**
Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 24 - 48 hours.

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium sporogenes</em> ATCC 11437</td>
<td>50 -100</td>
<td>good - luxuriant</td>
</tr>
</tbody>
</table>
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Technical Data

Clostridium sporogenes  ATCC 19404
50 -100       good - luxuriant

Bacteroides vulgatus ATCC 8482
50 -100       good - luxuriant

Bacteroides fragilis ATCC 23745
50 -100       good - luxuriant

Clostridium perfringenes ATCC 13124
50 -100       good - luxuriant

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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