Fermentation Medium Base for C. perfringens

Fermentation Medium Base for C. perfringens is a basal medium recommended for determination of fermentation reaction of \textit{Clostridium perfringens} with added carbohydrate.

**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>Peptone, special</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>0.250</td>
</tr>
<tr>
<td>Agar</td>
<td>2.000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 22.25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense 9 ml amounts in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Before use heat in boiling water or free flowing steam for 10 minutes to remove dissolved oxygen and add 1 ml of 1% sterile Salicin and Raffinose solution in separate tubes.

**Principle And Interpretation**

Contamination of foods with clostridia is largely derived from soil (1) and is usually responsible for \textit{Clostridium perfringens} food poisoning. A heat labile enterotoxin produced by sporulating cells induces the major symptoms of diarrhea in perfringens poisoning. Although the enterotoxin is not preformed in the foods, the foods in which conditions are favorable for sporulation may contain enterotoxin (2, 3). Therefore \textit{Clostridium} are common food contaminations responsible for spoilage of canned foods, chill stored products etc (4).

Fermentation Medium base for C. perfringens was formulated by Spray (5) and is recommended by APHA (6) for determination of fermentation reaction of \textit{C. perfringens}. This medium helps in identification of \textit{C. perfringens} from other \textit{Clostridium} species.

Casein enzymic hydrolysate and peptone special provide the necessary growth nutrients. Sodium thioglycollate creates low oxygen tension required in the medium to facilitate the growth of anaerobic organisms.

Inoculate about 2 gram of food sample into 15 to 20 ml of Chopped Liver Broth (M606). Incubate at 35-37°C for 20-24 hours. Streak Tryptose Sulphite Cycloserine (T.S.C.) Agar Base (M837) containing Egg Yolk Emulsion (FD045) to obtain presumptive \textit{Clostridium perfringens} @. Select representative black colonies and inoculate Fluid Thioglycollate Medium (M009). Incubate at 35-37°C for 18-24 hours. Perform gram staining and isolate on Tryptose Sulphite Cycloserine (T.S.C.) Agar Base (M837). Incubate anaerobically at 35-37°C for 18-24 hours to obtain isolated colonies. The Fluid Thioglycollate Medium (M009) tubes can be further used to confirm \textit{C. perfringens} by performing biochemical identification including carbohydrate fermentation. \textit{C. perfringens} can be differentiated from other clostridia on the basis of acid production from carbohydrates. To test acid, transfer 1 ml of culture from Fermentation Medium Base for \textit{C. perfringens} (containing Salicin / Raffinose) to a test tube and add 2 drops of 0.04 % bromothymol blue. A yellow colour indicates acid production. Salicin is rapidly fermented by Clostridia other than \textit{C. perfringens}, while \textit{C. perfringens} produces acid from raffinose within 3 days, which is not shown by other species.

**Quality Control**

**Appearance**

Cream to yellow homogeneous free flowing powder

**Gelling**
Semisolid, comparable with 0.2% Agar gel

**Colour and Clarity of prepared medium**
Light amber coloured, clear solution without any precipitate

**Reaction**
Reaction of 2.22% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH**
7.20-7.60

**Cultural Response**
Cultural characteristics observed under anaerobic condition with added 1% Salicin and Raffinose solutions in 2 separate tubes containing media after an incubation at 35-37°C for 24-72 hours.(Acid production is tested by addition of 0.04% Bromothymol blue)

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Salicin (24 hours)</th>
<th>Raffinose (72 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium paraperfringens</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>acid and gas production</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens ATCC 12924</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>acid production, yellow colour</td>
<td></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**

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