Perfringens Agar Base (T.S.C.)

**Intended use**
Perfringens Agar Base (T.S.C.) recommended for the enumeration of *Clostridium perfringens* from food. The composition and performance criteria of this medium are as per the specifications laid down in ISO 7937:1985.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>15.000</td>
</tr>
<tr>
<td>Soya peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium metabisulphite</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)**: 7.6±0.2

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

**Directions**
Suspend 21 grams in 500 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 add rehydrated contents of one vial of TSC Supplement (FD014). Alternatively if fluorogenic detection is desired add rehydrated contents of Clostridium perfringens supplements (FD243). Mix well before pouring into sterile Petri plates.

**Principle And Interpretation**
Tryptose Sulphite Cycloserine Agar (TSC) was originally formulated by Harmon et al (1) for the enumeration of *C. perfringens* from food. TSC Agar has been documented as one of the most useful media for the quantitative recovery of *C. perfringens* while suppressing growth of other facultative anaerobes (2). Perfringens Agar Base is also recommended by APHA (3). Perfringens Agar Base (M837I) is recommended for enumeration of *C. perfringens* from foods by ISO Committee (4).

Tryptose, soya peptone, yeast extract, provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and trace elements essential for clostridial growth. Sodium metabisulphite and ferric ammonium citrate act as an indicator of sulphite reduction, indicated by black coloured colonies. D-cycloserine (FD014) help in the selective isolation of *C. perfringens* by inhibiting accompanying flora. Homogenized food samples can be directly streaked on the surface of plates or can be pre-enriched in Cooked Meat Medium (M149) before streaking.

**Type of specimen**
Food and animal feed samples.

**Specimen Collection and Handling**:
For food and animal feed samples, follow appropriate techniques for sample collection and processing as per guidelines (4). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**:
In Vitro diagnostic Use only. 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.

**Limitations**:
1. Some species of Clostridia may show poor growth. Preenrichment may be required.

**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
Light yellow to brownish yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Amber coloured clear to slightly opalescent gel.
Reaction
Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.6±0.2
pH
7.40-7.80
Cultural Response
Cultural characteristics observed under anaerobic condition with added TSC Supplement (FD014) and Egg Yolk Emulsion (FD045), after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Sulphite Reduction</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens ATCC</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>positive, blackening of medium</td>
<td>Positive Reaction</td>
</tr>
<tr>
<td>Clostridium sordellii ATCC</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order 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 (5,6).

Reference
4. International Organization for Standardization (ISO- 7937:2004) : Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of Clostridium perfringens - Colony count technique
5. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.