Trytome Yeast Sodium Sulphite Agar Base

**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>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>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>7.6±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 21 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. Aseptically add the rehydrated contents of one vial of TSC Supplement (FD014). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Tryptose Sulphite Cycloserine Agar (TSC) was originally formulated by Harmon et al (2) for the enumeration of *Clostridium 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 (3). Perfringens Agar Base is also recommended by APHA (4). Tryptone Yeast Sodium sulphite Agar Base has been recommended by the ISO Committee for the isolation of *C. perfringens* from water samples using membrane filtration technique (4).

Tryptone, Soya peptone and yeast extract provide nitrogenous compounds, carbon, long chain amino acids, sulphur, 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.

The water sample to be tested is filtered through 0.45 micron filter membrane and the membrane filter is then placed on Tryptone Yeast Sodium sulphite Agar Base and incubated anaerobically at 43-45°C for 18-24 hours. Sulfite reacts with ferric salt to produce sulfide which results in production of black or grey to yellow brown colonies.

Confirmatory test: Smear some growth of 24 hours old culture of *Clostridium perfringens* from Blood Agar / Columbia Agar Base / Tryptone Soya Agar (incubated anaerobically at 34-38°C) on the filter paper. Add 2-3 drops of Acid phosphatase Reagent (R096) on to the colonies of filter paper, observe for appearance of strong purplish colour developed within 3-4 min which is positive reaction for *Clostridium perfringens*.

**Type of specimen**

Water samples

**Specimen Collection and Handling**

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

---

Please refer disclaimer Overleaf.
Warning and Precautions:
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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:
1. For further identification confirmatory test is highly recommended.

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 forms in Petri plates.

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) after an incubation at 43-45°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>Acid phosphatase test*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em> ATCC 12924</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><em>Clostridium perfringens</em> ATCC 13124</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>positive, blackening of medium</td>
<td>Positive Reaction</td>
</tr>
</tbody>
</table>

Key: (*) : Acid phosphatase test - Positive reaction : Development of purplish colour within 3-4 minutes on addition of R096 -(Acid phosphatase reagent).

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


