Brilliant Green HiCynth™ Agar Base, Modified  

**Intended Use:**
Brilliant Green HiCynth™ Agar (Modified) is used for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces and other materials.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.5*</td>
<td>3.000</td>
</tr>
<tr>
<td>HiCynth™ Peptone No.4*</td>
<td>10.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.080</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.0125</td>
</tr>
<tr>
<td>Agar</td>
<td>20.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 29.0 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. AVOID OVERHEATING. Cool to 45-50°C. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (FD068). Mix well before pouring into sterile Petri plates.

**Principle And Interpretation**

*Salmonella* species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. (8) and further modified by Kauffmann (7). It is also recommended by APHA (9,10) FDA (2) and described in EP, BP and IP (4,11,12). Brilliant Green HiCynth™ Agar (Modified) is prepared by completely replacing animal based peptones with chemically defined peptones to avoid BSE/TSE/GMO risks associated with animal peptones.

This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. The medium contains HiCynth™ Peptone No.4 and HiCynth™ Peptone No.5 as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulfacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.

**Type of specimen**
Clinical: Faeces; Food& dairy samples; Water samples; Pharmaceutical samples.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3,9,13).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(2). 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. Though this medium is selective for *Salmonella* other species of *Enterobacteriaceae* may grow.
2. *Salmonella* Typhi and *Shigella* species may not grow on this medium.
3. Moreover *Proteus, Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive *Salmonella* isolates.

**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 light pink homogeneous free flowing powder

**Gelling**

Firm, comparable with 2.0% agar gel.

**Colour and Clarity of prepared medium**

Greenish brown clear to slightly opalescent gel forms in Petriplates

**Reaction**

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

**pH**

6.70-7.10

**Cultural Response**

Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50 -100</td>
<td>none-poor</td>
<td>0 -10 %</td>
<td>yellowish green</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739 (00012*)</td>
<td>50 -100</td>
<td>none-poor</td>
<td>0 -10 %</td>
<td>yellowish green</td>
</tr>
<tr>
<td><em>Escherichia coli</em> NCTC 9002</td>
<td>50 -100</td>
<td>none-poor</td>
<td>0 -10 %</td>
<td>yellowish green</td>
</tr>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>25923 (00034*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50 -100</td>
<td>fair-good</td>
<td>30 -40%</td>
<td>reddish pink</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50 -100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pinkish white</td>
</tr>
<tr>
<td>Salmonella Enteritidis ATCC 13076 (00030*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>pinkish white</td>
</tr>
<tr>
<td>Salmonella Abony NCTC 6017 (00029*)</td>
<td>50 -100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pinkish white</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.
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. 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

In vitro diagnostic medical device

CE Marking

Storage temperature

10°C

30°C

Do not use if package is damaged

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