Bismuth Sulphite Agar

Intended Use:
Recommended for the selective isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water supplies, food etc.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>HM Peptone B #</td>
<td>5.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>5.000</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Bismuth sulphite indicator</td>
<td>8.000</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>20.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.7 ± 0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

# - Equivalent to Beef Extract

Directions

Suspend 52.33 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. DO NOT STERILIZE IN AUTOCLAVE or by fractional sterilization since overheating may destroy the selectivity of the medium.

The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into sterile Petri plates.

Principle And Interpretation

The Salmonellae constitute the most taxonomically complex group of bacteria among *Enterobacteriaceae* (13). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of *S. Typhi* (2). Four clinical types of *Salmonella* infections may be distinguished (10) namely gastroenteritis, bacteremia or septicemia, enteric fever and a carrier state. Of the various media employed for the isolation and preliminary identification of Salmonellae, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive (4).

Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium (15-17). It is also recommended by various Associations (2,3,5,9,11,12) for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food and other products.

*S. Typhi, S. Enteritidis and S. Typhimurium* typically grow as black colonies with a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. *Salmonella Paratyphi A* grows as light green colonies. Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms.

Peptone and HM Peptone B serve as sources as carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. Clinical samples can be directly used to inoculate Bismuth Sulphite Agar. In case of food samples, pre enrichment of the sample is done prior to inoculation.

Type of specimen

Clinical samples: faeces, urine, blood and other pathological material, foodstuff, water samples.
Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3,12).
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. 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. **DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM**, as it destroys the selectivity of the medium.
2. **S.Typhi and S.Arizonae** exhibit typical brown colonies, with or without metallic sheen.
3. **This medium is highly selective and must be used in parallel with less selective media for isolation.**
4. With certain **Salmonella** species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies.
5. **Shigella** species are mostly inhibited on this medium; exceptions being **S. flexneri** and **S. sonnei** (9)
6. Some **Salmonella** like **S. Sendai**, **S. Berta**, **S. Gallinarum**, **S. Abortus-equi** are also inhibited (9).

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 greenish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Greenish yellow coloured, opalescent with flocculent precipitate forms in Petri plates.

Reaction
Reaction of 5.23% w/v aqueous solution at 25°C. pH : 7.7±0.2

pH
7.50-7.90

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

<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><em>Klesiella aerogenes ATCC 13048 (00175</em>)</em>*</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>brown-green (depends on the inoculum density)</td>
</tr>
<tr>
<td><em><em>Enterococcus faecalis ATCC 29212 (00087</em>)</em>*</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td>brown-green (depends on the inoculum density)</td>
</tr>
<tr>
<td><em><em>Escherichia coli ATCC 25922 (00013</em>)</em>*</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>brown-green (depends on the inoculum density)</td>
</tr>
<tr>
<td><em><em>Salmonella Enteritidis ATCC 13076 (00030</em>)</em>*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>black with metallic sheen</td>
</tr>
<tr>
<td><strong>Salmonella Typhi ATCC 6539</strong></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>black with metallic sheen</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Salmonella Typhimurium
ATCC 14028 (00031*)
50-100 good-luxuriant >=50% black with metallic sheen

Shigella flexneri ATCC 12022 (00126*)
50-100 none-poor <=10% brown

Escherichia coli ATCC 8739 (00012*)
50-100 none-poor <=10% brown to green, depends on inoculum density

Escherichia coli NCTC 9002 50-100 none-poor <=10% brown to green (depends on inoculum density)

Salmonella Abony NCTC 6017 (00029*)
50-100 good-luxuriant >=50% black with metallic sheen

Key : *Corresponding WDCM numbers.
#- Formerly known as Enterobacter aerogenes

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (6,7).

Reference
17. Wilson and Blair, 1931, J. Hyg., 31:138

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In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg, Mumbai-86, MS, India

CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands,
www.cepartner4u.eu

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