Bismuth Sulphite HiVeg™ Agar Modified

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>HiVeg™ peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>HiVeg™ extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>5.000</td>
</tr>
<tr>
<td>Disodium hydrogen 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>0.016</td>
</tr>
<tr>
<td>Agar</td>
<td>12.700</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.6±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

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 the sterile Petri plates.

Principle And Interpretation

The *Salmonellae* constitute the most taxonomically complex group of bacteria among *Enterobacteriaceae* (12). 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 (8). 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, Modified is a modification of the original formulation of Wilson and Blair Medium (14). It is also recommended for the isolation of *Salmonella* Typhi and other *Salmonella* (2, 5). Bismuth Sulphite HiVeg™ Agar Modified is same as Bismuth Sulphite Agar Modified except that the animal based peptones are completely replaced by vegetable peptones to avoid the BSE/TSE risks associated with the animal peptones.

*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 HiVeg™ Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. *Shigella* species are mostly inhibited on this medium; exceptions being *S.* flexneri and *S.* sonnei (9) and also some *Salmonella* like *S.* Sendai, *S.* Berta, *S.* Gallinarum, *S.* Abortus-equ are inhibited (9). Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action toward gram-positive organisms and coliforms.

HiVeg™ peptone and HiVeg™ extract serve as sources of carbon, nitrogen, vitamins and essential growth factors. Dextrose (Glucose) is the carbon source. Disodium hydrogen 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 HiVeg™ Agar. In case of food samples, pre enrichment of the sample is done prior to inoculation.
**Type of specimen**
Clinical samples - Blood, faeces and other pathological specimen; Food and dairy samples; 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 (1,11,13).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).
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 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.
6. Some Salmonella like S. Sendai, S. Berta, S. Gallinarum, S. Abortus-equi are also inhibited.

**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 1.27% Agar gel.

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

**Reaction**
Reaction of 4.0% w/v aqueous solution at 25°C, pH: 7.6±0.2

**pH**
7.40-7.80

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

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<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of Colony</th>
</tr>
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<tbody>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>brown-green (depends on inoculum density)</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212 (00087*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td>brown-green (depends on inoculum density)</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>brown-green (depends on inoculum density)</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 19430</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>black with metallic sheen</td>
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### Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C, but not for more than 2 days as after which dye oxidizes to give green medium that could be inhibitory to some Salmonellae. 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


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<thead>
<tr>
<th><strong>Salmonella Paratyphi B</strong></th>
<th><strong>Shigella flexneri ATCC 12022</strong></th>
<th><strong>Salmonella Typhimurium ATCC 14028</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 8759</td>
<td>(00030*)</td>
<td>(00031*)</td>
</tr>
<tr>
<td>50-100</td>
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<td>brown</td>
<td>black with metallic sheen</td>
</tr>
</tbody>
</table>

Key : (*) - Corresponding WDCM numbers.

(#{})- Formerly known as *Enterobacter aerogenes*
Disclaimer:

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