**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>20.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>1.000</td>
</tr>
<tr>
<td>Mannitol</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>0.500</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
</tbody>
</table>

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

**Principle And Interpretation**

Hajna (4) developed Gram Negative (GN) Broth as an enrichment medium for recovery of *Salmonella* and *Shigella* from clinical and non-clinical specimens such as urine, blood clots, throat swabs, swabs from eating and drinking utensils etc (4, 5). GN Broth, Hajna is also recommended by APHA (11) for the microbiological examination of foods. Croft and Miller isolated more strains of *Shigella* from rectal swabs using this medium (1). Taylor and Schelhart showed the superiority of GN Broth to selenite enrichment media for isolation of *Shigella* (11). Hajna (5,6) also suggested the enrichment of organisms from rectal swabs in this medium 1-6 hours before plating on solid media.

The medium contains tryptose, which provides amino acids and other nitrogenous substances to support bacterial growth. The combination of sodium citrate and sodium deoxycholate inhibit gram-positive and some gram-negative bacteria such as coliforms. Phosphates serve as a buffering system. Sodium chloride maintains osmotic equilibrium. The higher concentration of mannitol over dextrose limits the growth of Proteus and enhances growth of mannitol fermenting *Salmonella* and *Shigella*. *Proteus, Pseudomonas* and coliforms do not overgrow *Salmonella* and *Shigella* in GN Broth during the first 6 hours of incubation. This enrichment broth should be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens (9,10,11).

GN Broth, Hajna should be inoculated directly with the specimen. In case of stool specimens, approximately 1 gram should be used for inoculation. Appropriate references for processing of clinical and food samples should be followed (2,3,10,11). After incubation of 6-8 hours and again after 24 hours, sub culturing on selective agar media should be carried out (9).

**Type of specimen**

Clinical samples - Blood

**Specimen Collection and Handling:**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8). 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. Further isolation and biochemical tests must be performed for confirmation.

**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**
Sterile GN Broth, Hajna in bottle.

**Colour**
Light amber coloured solution.

**Quantity of medium**
100 ml of medium in bottle

**pH**
6.80 - 7.20

**Sterility Testing**
Passes release criteria

**Cultural Response**
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours. Recovery is observed on MacConkey Agar (M081)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth in GN broth</th>
<th>Growth after 24 hours on MacConkey Agar</th>
<th>Colour of colony on MacConkey Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>pink-red with bile ppt</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 50-100 (00009*)</td>
<td>none-poor</td>
<td>none-poor</td>
<td>none-poor</td>
<td>pale pink-red</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> ATCC 19433 (00009*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC</em> 27853 (00025*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium ATCC</em> 14028 (00031*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Shigella flexneri</em> ATCC 12022 (00126*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
</tbody>
</table>

Key: (*) corresponding WDCM numbers

**Storage and Shelf Life**
On receipt store between 15-25°C. 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 (7,8).

**Reference**

*Please refer disclaimer Overleaf.*

In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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