GN Broth, Hajna

Intended use
Recommended for selective enrichment of gram-negative enteric organisms.

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</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 phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 39.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes or flasks as desired. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes. AVOID EXCESSIVE HEATING.

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* (12). 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*. 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).

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.

Please refer disclaimer Overleaf.
**Limitations:**
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**
Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**
Light amber coloured, clear to slightly opalescent solution in tubes.

**Reaction**
Reaction of 3.9% w/v aqueous solution at 25°C. pH: 7.0±0.2

**pH**
6.80-7.20

**Cultural Response**
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

<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</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 19433 (00009*)</td>
<td>non-poor</td>
<td>none-poor</td>
<td>pale pink-red</td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> ATCC 25933</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853 (00025*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 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**
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. 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.

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**Disclaimer:**

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