Hugh Leifson Medium

**Intended use**

Hugh Leifson Medium is used to distinguish between anaerobic and aerobic breakdown of carbohydrate (glucose).

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.000</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.050</td>
</tr>
<tr>
<td>Agar</td>
<td>2.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 19.35 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes in duplicate for aerobic and anaerobic fermentation. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medium in an upright position.

**Principle And Interpretation**

Hugh Leifson Medium was formulated by Hugh and Leifson (1). They described the taxonomic significance of fermentative and oxidative metabolism of carbohydrates in gram-negative intestinal bacteria.

There are two ways of utilizing carbohydrates by microorganisms, namely fermentation and oxidation. This property may be frequently used for the differentiation of some bacteria.

The medium contains a high concentration of carbohydrate and low concentration of peptone to avoid the possibility of an aerobic organism utilizing peptone and producing an alkaline condition which would neutralize slight acidity produced by an oxidative organism (2,3). Dipotassium phosphate promotes fermentation and acts as pH controlling buffer. Agar concentration enables the determination of motility and aids in distribution of acid throughout the tube produced at the surface of medium. The tubes for aerobic and anaerobic fermentation are inoculated and the agar surface of one tube of duplicate is covered with layer of sterile paraffin oil, about 25 mm thickness and incubated at 37°C. Oxidative organisms produce acid in unsealed tube with little or no growth and no acid formation in sealed tube while fermentative organisms produce acid in both sealed and unsealed tubes. If acid is produced, it is indicated by change in colour from greenish blue to yellow throughout the medium. Liquid paraffin tube used should be dry sterilized at 160-170°C for 2 hours. Wet sterilization with high pressure is not sufficient for the purpose.

**Type of specimen**

Clinical samples - Blood ; Food and dairy samples ; Water samples

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,9).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6) 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:
This medium is general purpose medium and may not support the growth of fastidious organisms.

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 bluish green homogeneous free flowing powder
Gelling
Semisolid, comparable with 0.2% Agar gel.

Colour and Clarity of prepared medium
Greenish blue coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 1.94% w/v aqueous solution at 25°C. pH : 6.8±0.2
pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Motility</th>
<th>Aerobic fermentation</th>
<th>Anaerobic fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>50-100</td>
<td>positive, growth away from stabline causing turbidity</td>
<td>acid (yellow) and gas production</td>
<td>acid (yellow) and gas production</td>
</tr>
<tr>
<td>ATCC 13048</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>50-100</td>
<td>positive, growth away from stabline causing turbidity</td>
<td>acid (yellow) and gas production,</td>
<td>acid (yellow) and gas production</td>
</tr>
<tr>
<td>ATCC 25922</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>50-100</td>
<td>positive, growth away from stabline causing turbidity</td>
<td>acid (yellow) production</td>
<td>unchanged (green) or alkaline (blue)</td>
</tr>
<tr>
<td>ATCC 27853</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
<td>50-100</td>
<td>positive, growth away from stabline causing turbidity</td>
<td>acid (yellow) and gas production</td>
<td>acid (yellow) and gas production</td>
</tr>
<tr>
<td>ATCC 6539</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Shigella sonnei</em></td>
<td>50-100</td>
<td>negative, growth along the stabline, surrounding medium</td>
<td>acid (yellow) production</td>
<td>acid (yellow) and gas production</td>
</tr>
<tr>
<td>ATCC 25931</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

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. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Please refer disclaimer Overleaf.
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

Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.