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 (Dextrose)</td>
<td>10.000</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar</td>
<td>3.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 20.33 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 10 lbs pressure (115°C) for 20 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.

It is recommended by BIS (2) for the isolation and cultivation of *Vibrio cholerae* and other *Vibrio* species which cause food poisoning.

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 (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. 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.

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.

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 2.03% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30

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><strong>Enterobacter aerogenes</strong></td>
<td>50-100</td>
<td>positive, growth away from stabline causing</td>
<td>acid (yellow) and gas production,</td>
<td>acid (yellow) and gas production,</td>
</tr>
<tr>
<td>ATCC 13048 (00175*)</td>
<td></td>
<td>turbidity</td>
<td>positive reaction</td>
<td>positive reaction</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>50-100</td>
<td>positive, growth away from stabline causing</td>
<td>acid (yellow) and gas production,</td>
<td>acid (yellow) and gas production,</td>
</tr>
<tr>
<td>ATCC 25922 (00013*)</td>
<td></td>
<td>turbidity</td>
<td>positive reaction</td>
<td>positive reaction</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>50-100</td>
<td>positive, growth away from stabline causing</td>
<td>acid (yellow) production,</td>
<td>unchanged (green) or alkaline (blue),</td>
</tr>
<tr>
<td>ATCC 27853 (00025*)</td>
<td></td>
<td>turbidity</td>
<td>positive reaction</td>
<td>negative reaction</td>
</tr>
<tr>
<td><strong>Salmonella Typhi</strong></td>
<td>50-100</td>
<td>positive, growth away from stabline causing</td>
<td>acid (yellow) and gas production,</td>
<td>acid (yellow) and gas production,</td>
</tr>
<tr>
<td>ATCC 6539</td>
<td></td>
<td>turbidity</td>
<td>positive reaction</td>
<td>positive reaction</td>
</tr>
<tr>
<td><strong>Shigella sonnei</strong></td>
<td>50-100</td>
<td>negative, growth along the stabline,</td>
<td>acid (yellow) production,</td>
<td>acid (yellow) and gas production,</td>
</tr>
<tr>
<td>ATCC 25931</td>
<td></td>
<td>surrounding medium</td>
<td>positive reaction</td>
<td>positive reaction</td>
</tr>
</tbody>
</table>

Key- (*) coresponding WDCM folders

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 inorder 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


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In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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Please refer disclaimer Overleaf.
Disclaimer:

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