Dubos HiVeg™ Broth Base

Intended Use:
Recommended for preparation of liquid medium for rapid cultivation of pure cultures of *Mycobacterium tuberculosis* and related microorganisms.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ hydrolysate</td>
<td>0.500</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>2.000</td>
</tr>
<tr>
<td>Polysorbate 80 (TWEEN 80)</td>
<td>0.200</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.050</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.010</td>
</tr>
<tr>
<td>Calcium chloride anhydrous</td>
<td>0.0005</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>0.0001</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>0.0001</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.6±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 1.3 grams in 180 ml purified / distilled water containing 10 ml glycerol. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 20 ml sterile bovine albumin V or sterile serum or 1 vial of sterile Albumin Glucose Supplement (FD201) to each 180 ml of broth base.

**Principle And Interpretation**

Tuberculosis remains a major global public health problem worldwide. *Mycobacterium tuberculosis*, the causative agent of tuberculosis in man, is carried in airborne particles known as droplet nuclei that are generated when patients with pulmonary tuberculosis cough. Infections occur when a susceptible person inhales the droplet nuclei containing the bacterium (6). Dubos Broth is formulated as per Dubos, et al (1), and is a modification of the medium originally formulated by Dubos and Davis (2) and Dubos and Middlebrook (3). Dubos HiVeg™ Broth Base is same as Dubos Broth Base except that the animal peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with animal peptones. Dubos media contain HiVeg™ hydrolysate and L-asparagine as sources of nitrogen. Polysorbate 80, an oleic acid ester also acts as a surfactant. It therefore supplies the essential fatty acids for the replication of Mycobacteria and also increases the growth by dispersing the bacilli. The phosphates (together with calcium chloride) buffers the media as well as serve as sources of phosphates. Magnesium sulphate, zinc sulphate, copper sulphate and ferric ammonium citrate provide trace metals and sulphates. Bovine albumin binds the free fatty acids, which may be toxic to Mycobacteria. Albumin is heat treated to inactivate the lipase, which may release fatty acids from Polysorbate 80 incorporated in the medium. Dubos HiVeg™ Broth Base enriched with serum will generally initiate growth from smaller inocula and yield more luxuriant growth than the basal medium enriched with albumin V. Growth is generally more granular with the serum enrichment, while it is more diffused with albumin enrichment. Maximum care should be taken while handling Mycobacterial cultures, as they are highly infectious.

**Type of specimen**

Clinical samples : Sputum

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). 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. This medium is general purpose medium and may not support the growth of fastidious organisms.
2 Proteolytic contaminants cause localized or complete digestion of medium.

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 beige homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured, clear solution without any precipitate

Reaction
Reaction of 0.65% w/v aqueous solution with 1% glycerol at 25°C. pH : 6.6±0.2

pH
6.40-6.80

Cultural Response
Cultural characteristics observed with added Albumin Glucose Supplement (FD201) or sterile bovine albumin V or sterile serum after an incubation at 35-37°C for 2-6 weeks with 5-10% CO₂.

Organism Growth
Mycobacterium avium ATCC 25291 luxuriant
Mycobacterium gordonae ATCC 14470 luxuriant
Mycobacterium kansasii ATCC 12478 luxuriant
Mycobacterium smegmatis ATCC 14468 luxuriant
M. tuberculosis H37 Rv ATCC 25618 luxuriant

Storage and Shelf Life
Store below 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.

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 (4,5).
Reference


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