**Hoyle Medium Base**

**Intended use**
Hoyle Medium Base is a highly selective medium used for the isolation and differentiation of *Corynebacterium diphtheriae* types.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>HM peptone B#</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.8±0.2</td>
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</tbody>
</table>

**Directions**
Suspend 40.0 grams in 940 ml purified/distilled water. Heat to boiling 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 50 ml of laked blood and 10 ml of 3.5% Potassium Tellurite Solution (FD047). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**
The most common disease caused by *Corynebacterium diphtheriae* is diphtheria, an acute communicable disease manifested by both local infection of the upper respiratory tract and the systemic effects of the toxin, which are most notable in the heart and peripheral nerves (6). Hoyle Medium Base, formulated by Hoyle (2), is the modification of the original formulation of Neill, for the isolation and differentiation of *C. diphtheriae*. This medium is not inhibitory to some mitis types of *Corynebacterium*, as the original formulation. Peptone and HM peptone B supply carbon, nitrogen substances, amino acids, vitamins and other essential growth nutrients. Potassium tellurite is a selective agent, which inhibits most of the normal flora of the upper respiratory tract except *Corynebacterium*. Hoyle's Medium is a highly selective medium and should be used in conjunction with a non-selective media such as Loeffler Serum Medium (M537) and Blood Agar Base (M089) with 10% horse blood (3). *C. diphtheriae* are usually present in small numbers permitting the formation of well isolated colonies. So, inoculation is done by directly rubbing the swab over the entire surface of the medium. Incubation should be carried out till 72 hours if the results are negative. To study the morphology, gentian violet staining is done. To demonstrate the characteristic morphology and staining reactions of *C. diphtheriae* by Neissers or Alberts method, it is advisable to use colonies from Loeffler Medium. The toxigenicity of *C. diphtheriae* strains can be determined by Eleks (1) method.

**Type of specimen**
Clinical samples - Blood

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). 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. Some species may show poor growth due to nutritional variations.

**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

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal Medium: Amber coloured, clear to slightly opalescent gel. After Addition of blood & Tellurite: Brownish red coloured opaque gel forms in Petri plates

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C: pH: 7.8±0.2

pH
7.60-8.00

Cultural Response
Cultural characteristics observed with added 50 ml of laked blood and tellurite solution, after an incubation at 35-37°C for 18-24 hours.

Organism  | Inoculum (CFU) | Growth | Recovery | Colony characteristics
--- | --- | --- | --- | ---
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)  | >=10⁴ | inhibited | 0% |  
|  |  |  |  |  
C. diphtheriae type intermedius 14779  | 50-100 | good-luxuriant >=50% | grey colonies with darker centers  
|  |  |  |  
Corynebacterium diphtheriae type mitis  | 50-100 | good-luxuriant >=50% | grey colonies with shining surface  
|  |  |  |  
Escherichia coli ATCC 25922 (00013*)  | >=10⁴ | inhibited | 0%  
|  |  |  |  
Enterococcus faecalis ATCC 50-100 29212 (00087*)  | 50-100 | good-luxuriant >=50% | black minute colonies  

Key: (*) Corresponding WDCM numbers.

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.

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 (3,4).

Reference
Disclaimer:

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