Hoyles Media Plate with supplements

Intended use
Recommended 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>3.5% Potassium Tellurite Solution (FD047).</td>
<td>10 ml</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.8±0.2</td>
</tr>
</tbody>
</table>

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

# - Equivalent to Beef extract

**Directions**

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

**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. 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 - Nasal swab, Throat swab, wound swab

**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 pack. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium

2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user’s unique requirement.

3. Further biochemical and serological tests must be carried out 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
Sterile Hoyles Media in 90 mm disposable plates.

Colour of medium
Amber coloured medium

Quantity of medium
25 ml of medium in 90 mm disposable plates.

pH
7.60-8.00

Sterility Test
Passes release criteria

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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colony characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis subsp. spizizenii ATCC 6653 (00003*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>C. diphtheriae type intermedia 14779</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>grey colonies with darker centers</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae type mitis</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>grey colonies with shining surface</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 50-100 29212 (00087*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>black minute colonies</td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

Storage and Shelf Life
On receipt store between 2-8°C 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
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