Cryptococcus Differential Agar

Cryptococcus Differential Agar is recommended for a differentiation of Cryptococcus species.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20.000</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.500</td>
</tr>
<tr>
<td>DL- Tryptophan</td>
<td>2.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>0.005</td>
</tr>
<tr>
<td>Trypan Blue</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 5.4±0.2

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

**Directions**

Suspend 44.04 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C and pour into sterile Petri plates.

**Principle And Interpretation**

Cryptococcus is the etiological agent of cryptococcosis, a systemic mycosis of humans and animals with a worldwide distribution. Cryptococcosis (earlier called European blastomycosis) commonly starts following inhalation of the organism, which is considered opportunistic infections as it affects mainly immunosuppressed individuals. (3)

This medium was based on the formulation of m-FDTG medium except the sugar fructose was replaced by glucose as it supported better growth of Cryptococcus species. Glucose supports growth as well as strong pigment production by nearly all C. gattii strains. C. gattii can while C. neoformans cannot assimilate D-tryptophan (1), thereby producing a brown diffusible pigment (4). Pigmentation is not apparent on the first day of growth but is usually noticeable after 5 days of incubation, intensity gradually increases with time after 2-3 weeks. (2)

Glycine serves as a sole source of carbon and nitrogen which is utilized by Cryptococcus gattii Cryptococcus laurentii and not by Cryptococcus neoformans. Salts in the medium help in pigment induction by D-tryptophan. Pigment production was more intense at 25-30°C as compared to 37°C. Dyes in media for the isolation of fungi have not been commonly utilized, although many such media are available for the isolation of bacteria. Trypan blue medium allows suspected C. neoformans colonies to be subcultured before mold overgrowth becomes a problem (5).

**Quality Control**

**Appearance**

Light yellow to yellow with bluish tinge homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Light blue coloured, opalescent gel with white precipitate forms in Petri plates

**Reaction**

Reaction of 4.4 % w/v aqueous solution at 25°C. pH : 5.4±0.2

**pH**

5.20-5.60

**Cultural Response**

M1814: Cultural characteristics observed after an incubation at 25-30°C for 5 to 6 days.
Organism | Inoculum (CFU) | Growth | Colony Characteristics
--- | --- | --- | ---
Cryptococcus neoformans ATCC 32045 | 50-100 | luxuriant | Light blue, dry colony
Cryptococcus laurentii ATCC 18803 | 50-100 | luxuriant | Brown, dry colony
Cryptococcus gattii ATCC MYA-4566 | 50-100 | luxuriant | Brown, mucoid colony

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

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