4. **ESCULEN BASE MEDIUM (EBM) pH 7.1**

   Dist. H₂O      1000 ml.
   Bacto Agar (Difco)  15 g.
   Dextrose (BBL)    5 g.
   Bacto-Peptone (Difco) 10 g.
   Esculin (Difco/BDH) 0.5 g.
   Difco Yeast Extract 1.0 g.

   Mix thoroughly to dissolve.
   Autoclave at 121°C/15 minutes

   Cool to 45°-50°C, aseptically remove 5.0-ml. agar, then add:

   2.5 ml. Gentamicin sulphate = 25,000 µg/litre
   2.5 ml. Chloramphenicol = 10,000 µg/litre

   Mix well and pour plates.
   Store in fridge.

**Gentamicin Sulphate Stock Solution (10,000 µg/ml)**

Vial contains 2.0 ml. (40 mg/ml) = 80,000 µg

Transfer contents of vial and make up to a volume of 8 ml. using phosphate buffer pH 8.0 (= 10,000 µg/ml). Distribute 3 ml. amounts into bijou bottles. Store at -20°C.

**Chloramphenicol Stock Solution (4,000 µg/ml)**
Purpose

Differential medium for isolation of Cryptococcus neoformans and also isolation medium for other fungi from contaminated specimens. Also provides presumptive identification of *C. neoformans*.

Principle

*C. neoformans* produces phenol oxidase enzyme that breaks down the substrate esculin, resulting in the production of a melanin-like pigment and the development of dark brown colonies. It takes about 48-72 hours for colonies to become brown. Other yeast colonies are cream to beige.

Rare strains of *C. neoformans* fail to produce pigmented colonies; also rarely yeasts other than *C. neoformans* produce dark colonies.

Quality Control

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Incubation Temperature</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcus neoformans</td>
<td>28°C</td>
<td>Brown pigment</td>
</tr>
<tr>
<td><em>C. laurentii</em></td>
<td>28°C</td>
<td>No brown pigment</td>
</tr>
<tr>
<td>(or <em>T. glabrata</em>)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References