Cooke Rose Bengal Agar Base

Intended Use:
Recommended for selective isolation and cultivation of fungi.

Composition***

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya Peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>10.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.500</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>0.035</td>
</tr>
<tr>
<td>Agar</td>
<td>20.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.0±0.2</td>
</tr>
</tbody>
</table>

***Formula adjusted, standardized to suit performance parameters

Directions
Suspend 36.54 grams in 1000 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. To increase the selectivity of the medium, add 35µg chlortetracycline per ml of the medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation
Cooke Rose Bengal Agar is a selective medium formulated as per Cooke (2,3). A variety of inhibitory agents have been used to inhibit bacteria in an attempt to isolate fungi from mixed flora. The Kingdom Fungi includes some of the most important organisms, both in terms of their ecological and economic roles. By breaking down dead organic material into simpler forms, they continue the cycle of elements through ecosystems. In addition, most vascular plants could not grow without a symbiotic association with fungi, or mycorrhizae, that inhabit their roots and supply essential nutrients. Other fungi provide numerous drugs (such as penicillin and other antibiotics), foods like mushrooms, truffles and morels, and the bubbles in bread, champagne, and beer (7). Waksman (11) described an acid medium consisting of peptone, dextrose, inorganic salts and agar for the isolation of fungi from soil. Cooke (2) used the Waksman medium without adjustment for isolation of fungi from sewage. It was discovered that soya pepton was particularly suitable for use in this medium and that the combination of chlortetracycline, or oxytetracycline, with rose bengal increased the selectivity of the medium.

Smith and Dawson (9) used rose bengal for the inhibition of bacteria in media which has almost neutral reaction concerned with retardation of the development of fungi. Martin (6) used 1: 30,000 Rose bengal and 30µg Streptomycin per ml and found that a wide variety of bacteria are inhibited at reactions between pH 5.5 to 6.5 without inhibiting fungi.

The medium should not be exposed to light as photo-degradation of rose bengal yields compounds that are toxic to fungi (1,8).

Microscopic examination coupled with biochemical testing using pure cultures is recommended for complete identification. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or partially inhibited.

Soya Peptone provides nitrogen, carbon and vitamins. Dextrose is an energy source. Rose bengal and chlortetracycline selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing moulds. Potassium dihydrogen phosphate provides buffering capability. Magnesium sulfate is a source of divalent cations.

Type of specimen
Food samples; Soil samples; Industrial fermentation samples.

Specimen Collection and Handling:
For soil samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (8).
For industrial fermentation samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions:**
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 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.

**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 light pink homogeneous free flowing powder

**Gelling**
Firm, comparable with 2.0% Agar gel

**Colour and Clarity of prepared medium**
Pink-red coloured, slightly opalescent forms in Petri plates

**Reaction**
Reaction of 3.65% w/v aqueous solution at 25°C. pH : 6.0±0.2

**pH**
5.80-6.20

**Cultural Response**
Cultural characteristics observed after an incubation at 25-30°C for 1-4 days.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth (Plain)</th>
<th>Recovery (Plain)</th>
<th>Growth with chlorotetra cycline</th>
<th>Recovery with chlorotetra cycline</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em> ATCC 10231 (00054*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> ATCC 9763 (00058*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>#<em>Aspergillus brasiliensis</em> ATCC 16404 (00053*)</td>
<td>50-100</td>
<td>good</td>
<td>good</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0%</td>
<td>inhibited</td>
<td>0%</td>
</tr>
</tbody>
</table>

Key :*Corresponding WDCM numbers.
# Formerly known as *Aspergillus niger*

**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.

Please refer disclaimer Overleaf.
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