Crystal Violet Lactose Agar

**Intended Use:**
Recommended for differentiation of pure cultures of pathogenic and nonpathogenic Staphylococci.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>HM peptone B #</td>
<td>3.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.0033</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td><strong>6.8±0.1</strong></td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**
# - Equivalent to Beef extract

**Directions**
Suspend 33 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. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**
Crystal Violet Lactose Agar was recommended by Chapman (1) for the differentiation of pure cultures of pathogenic from nonpathogenic strains of Staphylococci.

The toxicity of Staphylococci is estimated on the basis of their pigment production, haemolytic and coagulating characteristic. In the study of the correlation between haemolytic and coagulase activities, animal inoculation and other tests, Chapman and Berens (2, 3) reported that Staphylococci produced different coloured growths when cultured on Crystal Violet Agar. Haemolytic and coagulating strains produced purple to violet colour whereas non-hemolytic and non-coagulating strains produced white colonies after incubation. Crystal violet inhibits most of the gram-positive organisms and is markedly inhibitory to Staphylococci. A fair growth can be obtained at a 1: 300,000 concentration of the dye when the medium is inoculated heavily. So, this medium is used for study of pure cultures where a mass inoculation can be used rather than for primary isolation.

The media contains proteose peptone and HM peptone B as sources of carbon, nitrogen, vitamins and minerals. Lactose is the carbon and energy source.

**Type of specimen**
Pure isolate

**Specimen Collection and Handling:**
For pure isolate samples follow appropriate techniques for handling specimens as per established guidelines (2,3). 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. 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.
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 tan homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.3% w/v aqueous solution at 25°C. pH : 6.8±0.1

pH

6.70-6.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>purple</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp.</td>
<td>50-100</td>
<td>fair-good</td>
<td>30-40%</td>
<td>light yellow</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>50-100</td>
<td>fair - good</td>
<td>30-40%</td>
<td>purple/ very slightly yellow</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC</td>
<td>50-100</td>
<td>none - poor</td>
<td>0 - 10%</td>
<td>colourless</td>
</tr>
<tr>
<td>19615</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference


Disclaimer:

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