Milk Agar

Milk Agar is recommended for enumeration of bacteria in milk and milk products, rinse waters, ice creams etc.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Milk solids</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 24 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

The milk secreted in an uninfected cows udder is sterile. Contamination of this milk can occur during milking, cooling and storage (1). Milk is an excellent medium for bacteria, yeast and moulds. Their rapid growth can cause marked deterioration, spoiling the milk for liquid consumption or manufacture into dairy products. Human infection can occur by consumption of such contaminated milk or milk products. Milk Agar is recommended for performing plate count tests on milk, rinse waters and dairy products. It is formulated as per the official medium described by Department of Health Memo (2). It is also recommended by EUROGLACE (EEC Ice Cream Industries) for the examination of ice cream (3).

Peptic digest of animal tissue and yeast extract provide essential nutrients while milk solids are a source of casein. Dextrose is the carbon and energy source. Proteolytic bacteria will be surrounded by a clear zone, due to the conversion of casein into soluble nitrogenous compounds (4).

For milk, dilutions of 1/10, 1/100 and 1/1000 are prepared with 1/4 strength Ringer solution. 1 ml of each dilution is pipetted aseptically into sterile Petri plates to which 10 ml of sterile and cooled Milk Agar is added and mixed well. Plates should be poured within 15 minutes of dilution preparation. After solidification of medium the plates are allowed to stand for 1 hour before transferring to the incubator. Incubate at 35°C for 2 or 3 days at 30°C. Higher counts may be obtained after an incubation at 22°C and 30°C temperature rather than at 35°C (5, 6, 7). Count the colonies within 4 hours after the incubation and read it as per ml of sample.

**Quality Control**

**Appearance**
Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Light yellow coloured slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 2.4% w/v aqueous solution at 25°C, pH : 7.2±0.2

**pH**
7.00-7.40

**Cultural Response**
M163: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 48 hours.
### Organism

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis ATCC 6633</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Lactobacillus casei ATCC 9595</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Serratia marcescens ATCC 8100</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
</tbody>
</table>

### 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

4. Methods of Microbiological Examination for Dairy Purposes, Diluents, Media and Apparatus and their Preparation and Sterilization, BS4285, Sec. 1.2.