Litmus Milk is used for maintenance of Lactobacilli and for determining the action of bacteria on milk.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk powder</td>
<td>100.000</td>
</tr>
<tr>
<td>Litmus</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>0.500</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 6.8±0.2

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

**Directions**

Suspend 101 grams in 1000 ml distilled water, agitating continuously. Dispense 10 ml amounts into 15 x 150 mm tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. AVOID OVERHEATING.

**Principle And Interpretation**

Milk contains the carbohydrate lactose along with three main proteins i.e. casein, lactalbumin and lactoglobulin (1). Therefore an organism may exhibit one or several of the following metabolic properties in litmus milk, each specific for a particular species aiding bacterial identification. The various metabolic functions are lactose fermentation, litmus reduction, clot formation, peptonization (digestion) and gas formation (2). Litmus Milk is a differential medium used to determine different metabolic functions. Litmus Milk is also useful in the maintenance and propagation of lactic acid bacteria.

Litmus Milk is the most useful medium in dairy industry as it is a reliable indicator of bacterial action on milk (3). Litmus is a good indicator of acidity, alkalinity and its oxidation-reduction potential is useful in milk media with lower toxicity to microorganisms than bromocresol purple (4). Addition of 1% w/v dextrose and/or 5% w/v yeast extract to Litmus Milk accelerates the growth of some organisms, which cannot grow in plain Litmus Milk (3, 4, 5).

For detection of *Clostridium perfringens* in water, inoculate freshly heated tubes of Litmus Milk with various quantities of water and heat at 80°C for 10-15 minutes to destroy non-spore-forming organisms. Examine after every 24 hours for positive Stormy Clot reaction at 35°C for up to 5 days (6, 7). Anaerobiosis in Litmus Milk can be obtained by adding a small heated iron nail or 0.1 gram of reduced iron to the medium (8). Skim milk is the substrate, metabolized by particular species of bacteria in different ways. The actions of bacteria can be categorized as follows,

**ACID REACTION CAUSE**

1. Pink to red colour Fermentation of lactose of the milk and/or dextrose in milk.
2. Acid coagulation Lactic acid production, producing a casein curd in clear watery fluid.
3. Stormy clot Gas formation in coagulated casein curd.

**ALKALINE REACTION**

1. Blue colour of the Formation of basic amines or ammonia milk due to proteolysis.
2. Alkaline coagulation Paracasein formation from casein by enzyme renmin with a soft, blue clot.
3. Peptonization Digestion of casein, evident by clearing of the medium and dissolution of the clot REDOX REACTION 1. Decolourized medium Reaction of Litmus in the depths of (Similar to freshly the tube by reductase enzymes with autoclaved Litmus the resultant removal of oxygen to Milk) form the decolourized leucolitmus compound. Reactions obtained in this medium are not specific and further tests must be carried out.

**Quality Control**

**Appearance**

Pinkish purple to grey homogeneous free flowing powder may contain minute to small particles.
Colour and Clarity of prepared medium
Light purple coloured opaque milky solution

Reaction
Reaction of 10.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M609: Cultural characteristics observed after an incubation at 35-37°C for up to 14 days and record the reactions of various intervals during the incubation.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>good-luxuriant</td>
<td>stormy fermentation (gas)</td>
</tr>
<tr>
<td>ATCC 13124</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>good-luxuriant</td>
<td>acid clot (pink)</td>
</tr>
<tr>
<td>ATCC 11506</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>good-luxuriant</td>
<td>peptonization (clearing)</td>
</tr>
<tr>
<td>ATCC 27853</td>
<td></td>
<td></td>
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</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