



Litmus SM Broth

M609I

Intended Use:

Recommended for identification of *Lactobacillus*. The composition and performance criteria are in accordance with ISO 9232:2003(E) IDF 146:2003(E)

Composition**

ISO 9232:2003(E)IDF 146:2003(E)- Litmus SM Broth

Ingredients	g / L
Skim milk	100.000
Litmus powder	0.700
Final pH (at 25°C)	6.8±0.2

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**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 100.70 grams in 1000 ml purified / 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 SM Broth is recommended by ISO Committee(3).

Litmus Milk is the most useful medium in dairy industry as it is a reliable indicator of bacterial action on milk (4). 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 (5). 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 (4,5,6).

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 (7,8). Anaerobiosis in Litmus Milk can be obtained by adding a small heated iron nail or 0.1 gram of reduced iron to the medium (9). 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 rennin 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.

Type of specimen

Dairy samples, Water samples

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,11).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

(12) 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. Some strains may show poor growth due to nutritional variations.
2. Further biochemical and serological testing is required for complete identification.

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

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 10.07 % w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Growth	Reaction
<i>Clostridium perfringens</i> ATCC 13124 (00007*)	good-luxuriant	stormy fermentation (gas)
<i>Lactobacillus acidophilus</i> ATCC 11506	good-luxuriant	acid clot (pink)
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	good-luxuriant	peptonization (clearing)

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (13,14).

Reference

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