17158 Litmus Milk

For maintenance of lactobacilli and for determining the action of bacteria on milk.

Composition:

Ingredients	Grams/Litre
Skim milk powder	100.0
Litmus	0.5
Sodium sulphite	0.5

Final pH 6.8 +/- 0.2 at 25°C

Store granulated media between 10-30°C in tightly closed container and the prepared medium at 15-25°C. 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.

Appearance(color): Faint blue and Faint purple and Faint red, free flowing powder
Color and Clarity: Light purple coloured opaque milky solution

Directions:
Suspend 101 g in 1 litre distilled water, agitating continuously. Dispense 10 ml amounts into 15x150mm tubes and sterilize by autoclaving at 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 (4). 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 (5). Litmus Milk is a differential medium used to determine different metabolic functions.
It is also used to cultivate lactic acid bacteria associated with dairy products because litmus is a reliable indicator of action upon milk (1). 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 (2). 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 (1,2,3). In an acid solution litmus is red and under alkaline conditions it is blue When an organism ferments lactose, lactic acid is produced and the medium changes to a pink-red color. Some bacteria act on the nitrogenous substrates in the milk releasing ammonia and yielding a purplish-blue color. Some organisms can reduce litmus with reductase enzymes to a colorless compound called leucolitmus (7), this reaction only occurs only in the depths of the tubes and result as a white color at the bottom. Peptonization is visible by media clearance if the the milk proteins are digested. Organisms which can coagulate casein form a clot or a curd. This reaction happens if the organisms posses rennin which converts casein into paracasein. Whey, a clear liquid, is produced at the top of the thoroughly coagulated tube.
For detection of *Clostridium perfringens* in water, inoculate freshly heated tubes of Litmus Milk with various amount 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 (8,9). Anaerobiosis in Litmus Milk can be obtained by adding a small, heated iron nail or 0.1 gram of reduced iron to the medium (6). 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) forms the decolourized leucolitmus compound. Reactions obtained in this medium are not specific and further tests must be carried out

Cultural characteristics observed after an incubation at 35-37°C for up to 14 days 5-6 days.

Organisms (*ATCC*/*WDCM*)	Growth	Colony characteristics
Clostridium perfringens (*13124/00007*)	++/+++	Stormy fermentation(gas)
Lactobacillus acidophilus (11506/-)	++/+++	Acid clot(pink)
Pseudomonas aeruginosa (27853/00025)	++/+++	Peptonization (clearing)

References:

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