LITMUS MILK

Dehydrated culture medium

1 - INTENDED USE

Liquid medium for the differentiation of microorganism based on multiple metabolic reactions and for the maintenance of lactic acid bacteria.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Skim milk	100.00 g
Litmus	0.75 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Litmus Milk has been used for many years as a help in the differentiation of organisms (especially within the genus *Clostridium*) based on multiple metabolic reactions on milk, including fermentation, reduction, clot formation, digestion, and the formation of gas.[1] It is also useful in the maintenance and propagation of lactic acid bacteria.

Litmus incorporated in the medium is both a pH and an oxidation-reduction indicator. Milk contains lactose and three main proteins: casein, lactalbumin and lactoglobulin. At pH 6.5 the medium is pale blue coloured; when inoculated with lactose-fermenting microorganisms, which produce lactic acid and occasionally butyric acid, it becomes pinkish-red through the litmus reaction. Some bacteria, which do not ferment lactose but hydrolyse the casein make the medium alkaline with a fouls smell, turning the medium into a purplish-blue colour. Some organisms remove the oxygen in the medium by means of a reductase, with reduction of the litmus to the white leuco-base.

The peptonisation phenomenon is due to digestion of the casein, which manifests by clearing of the medium. Breakage of the coagulum indicates gas production by the inoculated strain.

Acid production from the fermentation of lactose is shown by a change in colour of the indicator, and, when much acid is produced, by the formation of a clot. But another form of clot may be produced by rennet; in this case the clot forms first and later, like the fibrin clot in blood, contracts and expresses a clear whey. In contrast the acid clot does not contract. When the bacterium also produces proteolytic enzymes, the clot may be peptonized.[2]

4 – DIRECTIONS FOR MEDIUM PREPARATION

Mix 100 g with a small quantity of cold purified water, making a smooth paste and add more purified water until a 10% mixture is obtained (100 g/L). Agitate the mixture continuously and dispense 5-10 mL amounts into suitable screwcap tubes. Sterilise by steaming (100°C) on three successive days for 60, 45 and 80 minutes. Alternatively, autoclave at 121°C for 5 minutes or at 110°C for 10 minutes. Overheating must be avoided to prevent caramelization.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-grey, fine, homogeneous, free-flowing powder.
Solution appearance	pale blue with pink-blue precipitate, opaque. During autoclaving litmus is reduced to a white coloured base however, upon cooling, oxygen is absorbed and the original colour returns.
Final pH at 20-25 °C	6.5 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Litmus Milk	Dehydrated medium	4016112	500 g (5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, screw-cap tubes, ancillary culture media and reagents.

8 - SPECIMENS

18-24 h pure broth culture.

9 - TEST PROCEDURE

1. Inoculate with 4 drops of a 18-24-hour pure broth culture.
1. Incubate at 35-37°C in aerobic atmosphere.
2. If *Clostridium* is suspected or anaerobiosis is desired, pour a layer of sterile mineral oil over medium surface immediately after inoculation.[1]
3. Observe daily for seven days for alkaline reaction (litmus turns blue), indicator reduction, acid clot, acid reaction (litmus turns pink), rennet clot, and peptonisation. Longer periods up to 14 days may be necessary.[1,2]
4. Record all changes.

10 - READING AND INTERPRETATION

Multiple changes can occur over the observation period.[1]

1. Pinkish-red: acid reaction; lactose and/or glucose fermented; red: lactose fermented, pink: glucose fermented
2. Purplish-blue: no fermentation of lactose, no change of litmus, same colour of uninoculated tube.
3. Blue: alkaline reaction; no fermentation of lactose, organism attacks nitrogenous substances to form ammonia or basic amines.
4. White: reduction of litmus to a white base by enzyme reductase.
5. Clot or curd formation: milk proteins coagulation due to either a precipitation of casein by acid formation or the conversion of casein in paracasein by the enzyme rennin resulting in a clear watery fluid called “whey”.
6. Digestion (peptonisation): milk protein digested; clearing of medium and dissolution of clot by digestion of casein
7. Gas production (H_2 and CO_2): bubbles in the medium and clot may be broken up.
8. Stormy clot: acid clot disrupted by an abundance of gas production

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C / 3-5 days / AN	acid, "stormy clot" coagulation
P. aeruginosa ATCC 27853	37°C / 3-5 days / A	peptonisation (clearing)
L. acidophilus ATCC 314	37°C / 3-5 days / A	acid, clot or curd

A: aerobic incubation; AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Litmus Milk, is tested for specific metabolic reactions by comparing the results with a previously approved Reference Batch.
Metabolic reactions are tested by inoculating the tubed medium with pure cultures of target organisms, incubating at 37°C for 3-5 days and recording the changes in the medium. Litmus Milk is tested with the following strains giving the following reactions: *C. perfringens* ATCC 13124 (acid reaction, pink colour, "stormy clot" coagulation), *L. acidophilus* ATCC 314 (acid reaction, pink colour and clotting), *E. faecalis* ATCC 19433 (acid reaction, reduction, white to colourless), *P. vulgaris* ATCC 9484 (no changes), *A. faecalis* ATCC 35655 (alkaline reaction, blue colour) *P. aeruginosa* ATCC 27853 (peptonisation, clearing).

13– LIMITATIONS OF THE METHOD

- A clot formation must be simply recorded as "clot". The differentiation between a clot and curd formation is not useful.
- Reactions observed in Litmus Milk are not sufficient to speciate; additional biochemical and serological tests must be performed.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the preparation process of tubed media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type, and the storage method applied (temperature and packaging). According to MacFaddin the tubed medium may be stored in screwcap tubes at +2°C /+8°C for 2-4 weeks.[1]

16 - REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. 3rd edition, edited and revised by Barrow GI and Feltham RKA. Cambridge University Press, 1993.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it