LITMUS MILK MEDIUM

INTENDED USE
Remel Litmus Milk Medium is a liquid medium recommended for use in qualitative procedures to determine the action of bacteria on milk and to maintain lactic acid bacteria.

SUMMARY AND EXPLANATION
Litmus Milk Medium is used to differentiate bacteria on the basis of lactose fermentation, casein hydrolysis and coagulation, gas production, and reduction of litmus. It is especially useful in species differentiation within the genus *Clostridium* and to differentiate *Streptococcus bovis* (no growth) from *Streptococcus equinus* (growth).[1] It is also used to cultivate lactic acid bacteria associated with dairy products because litmus is a reliable indicator of action upon milk.[2]

PRINCIPLE
Litmus is both an indicator of pH and of the oxidation-reduction (Eh) potential of the medium. Milk provides lactose, casein, lactalbumin, and lactoglobulin.[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. Organisms that reduce litmus cause the oxygen to be removed, leaving a leuco (white) base. Proteolytic enzymes produced by certain organisms, hydrolyze milk proteins and result in clot formation.[4] Casease production results in peptonization which causes digestion of the clot, exhibited by a watery clearing of the medium. The end result of lactose fermentation may be gas production (CO_2 and H_2). An abundance of gas breaks up an acid clot causing a reaction referred to as stormy fermentation. This may occur with certain anaerobic *Clostridium* species.

REAGENTS (CLASSICAL FORMULA)*
Skim Milk..... 100.0 g

Litmus..... 0.75 g
Demineralized Water ..... 1000.0 ml

pH 6.8 +/- 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Using a loop, inoculate Litmus Milk Medium from an 18-24 hour pure culture or a well-isolated colony of the test isolate.
2. If *Clostridium* is suspected or anaerobiosis is desired, add sterile reduced iron (e.g., iron powder, nails, paper clips, metal filings) to the tube or pour a layer of sterile mineral oil over the medium surface immediately after inoculation.[1]
3. Incubate in the appropriate atmosphere at 35-37°C for 18-24 hours. Longer periods up to 14 days may be necessary.[1]

INTERPRETATION OF THE TEST

(A)	Pinkish-red	Acid reaction, lactose fermentation
(ALK)	Blue	Alkaline reaction, no fermentation, organisms attack nitrogenous substances in the medium
(C)	Clot or curd formation	Milk protein coagulation
(D)	Digestion (peptonization)	Milk protein digested, clearing of medium
(G)	CO_2 and H_2	Bubbles in medium, clot may be broken up
(NF)	Purplish-blue	No fermentation, no change of indicator
(RED)	White	Reduction of litmus to a white leuco base by enzyme reductase
(SF)	Stormy Fermentation	Acid clot disrupted by an abundance of gas production

QUALITY CONTROL
All lot numbers of Litmus Milk Medium have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Escherichia coli ATCC® 25922	Ambient, up to 5 days @ 33-37°C	Acid
Clostridium innocuum ATCC® 14501	Anaerobic, up to 5 days @ 33-37°C	Alkaline, no clot, no digestion
Clostridium perfringens ATCC® 13124	Anaerobic, up to 5 days @ 33-37°C	Acid, clotting, digestion (SF)

LIMITATIONS
1. Do not attempt to differentiate between a clot and a curd formation.[1]
2. Positive and negative control organisms may be used to demonstrate the various reactions which may occur in Litmus Milk Medium.[1]

BIBLIOGRAPHY

1. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
2. Davis, J.G. 1935. J. Dairy Res. 6:121.
3. Cantarow, A. and B. Schepartz. 1962. Biochemistry. 3rd ed. W.B. Saunders Co., Philadelphia, PA.
4. Burrows, W. and J.W. Moulder. 1968. Textbook of Microbiology. 19th ed. W.B. Saunders Co., Philadelphia, PA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

ATCC® is a registered trademark of American Type Culture Collection.

IFU 61274, Revised April 4, 2013

Printed in U.S.A.

remel

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128