



## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

Litmus Milk is a medium for the maintenance of lactic acid bacteria and for the determination of bacterial action on milk.

### II PERFORMANCE TEST PROCEDURE

1. Loosen caps, boil the medium for 2 min and cool with tightened caps to room temperature before inoculation.
2. Inoculate representative samples with the cultures listed below.
  - a. For the clostridia, use cultures grown in Cooked Meat Medium. For the remaining organisms, use fresh agar cultures.
  - b. Immediately after inoculating each tube with clostridia, overlay with 1 mL of mineral oil.
  - c. Incubate tubes inoculated with aerobes with loosened caps at 35 ± 2 °C in an aerobic atmosphere; incubate tubes inoculated with anaerobes with tightened caps at 35 ± 2 °C. Examine for up to 7 days for reactions.
3. Expected Results

Organisms	ATCC®	Recovery	Reaction
* <i>Lactobacillus acidophilus</i>	314	Growth	Acid clot (pink)
* <i>Clostridium perfringens</i>	13124	Growth	Stormy fermentation (acid with strong evolution of gas) with clot
<i>Clostridium butyricum</i>	859	Growth	Stormy fermentation (acid with strong evolution of gas) with clot
<i>Clostridium sporogenes</i>	11437	Growth	Acid clot and peptonization
<i>Enterococcus faecalis</i>	29212	Growth	Acid and reduction (white to colorless)

\*Recommended organism strain for User Quality Control.

### III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes aerobically at 20–25 °C and 35–37 °C and examine after 5 days for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

Litmus Milk is used for the maintenance of lactic acid bacteria and as a differential medium for determining the action of bacteria on milk.

### V SUMMARY AND EXPLANATION

Litmus Milk has been used for many years for determining the metabolic activities of microorganisms in milk as an aid to the identification of bacterial species.<sup>1</sup> It is especially useful in species differentiation within the genus *Clostridium*. This medium is also of value in the maintenance and propagation of lactic acid bacteria.

### VI PRINCIPLES OF THE PROCEDURE

Skim milk is the substrate that particular species of bacteria attack in different ways to produce various metabolic products. Azolitmin serves as a pH indicator with a color range of pink (below pH 4.5) to purple (in middle of pH range) to blue (above pH 8.3) and also functions as an Eh (oxidation-reduction) indicator.<sup>2</sup>

The action of bacteria on milk can be categorized as follows:

1. No change (no carbohydrate fermentation and no change of litmus indicator).
2. Fermentation of lactose and/or dextrose in the milk with production of acid (pink color) including stormy fermentation (strong evolution of gas) by certain strains of *Clostridium*.
3. Action of proteolytic enzymes on lactalbumin with production of ammonia or basic amines resulting in an alkaline reaction (blue color).
4. Coagulation of casein as evidenced by the formation of a curd or clot. If the casein is converted to paracasein by the enzyme rennin, a clear, watery liquid called "whey" is produced at the top of a thoroughly coagulated tube.<sup>2</sup>
5. Peptonization due to digestion of the milk protein as evidenced by a clearing of the medium and dissolution of the clot.
6. Reduction of the litmus in the depths of the tube due to the action of reductase enzymes with the resultant removal of oxygen to form the decolorized leucolitmus compound.

### VII REAGENTS

#### Litmus Milk

Approximate Formula\* Per Liter Purified Water

Skim Milk .....	100.0 g
Azolitmin .....	0.5 g
Sodium Sulfit .....	0.5 g

\*Adjusted and/or supplemented as required to meet performance criteria.

#### Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

## VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

## IX PROCEDURE

**Material Provided:** Litmus Milk

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

Loosen caps, boil the medium for 2 min and cool with tightened caps to room temperature before inoculation.

Inoculate tubes of Litmus Milk with 18- to 24-h pure cultures. For the study of anaerobic organisms, overlay the medium with 1 mL of sterile mineral oil following inoculation. Incubate tubes at 35 ± °C in an aerobic atmosphere for up to 14 days and record reactions at various intervals during the incubation process.

**User Quality Control:** See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

## X RESULTS

Consult an appropriate text for the expected reactions for specific microbial species.<sup>3,4</sup>

## XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>1,3,5-7</sup>

## XII AVAILABILITY

Cat. No.	Description
221657	BBL™ Litmus Milk, Pkg. of 10 size K tubes

## XIII REFERENCES

1. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*, 11<sup>th</sup> ed. Mosby, Inc., St. Louis.
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4. Allen, S.D., C.L. Emery, and D.M. Lysterly. 2003. *Clostridium*, p. 835-856. In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
5. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
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7. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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