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

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

*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.

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. 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.

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.
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 Sulfite ......................................................... 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 anaerobic 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.\textsuperscript{3,4}

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.\textsuperscript{3,5-7}

XII AVAILABILITY

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>221657</td>
<td>BBL™ Litmus Milk, Pkg. of 10 size K tubes</td>
</tr>
</tbody>
</table>

XIII REFERENCES

Technical Information: In the United States contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.