

# FE 305 Experiment 3

## Bacteriological Examination of Milk and Milk Products

# Contents

- Coliform Count
- Methylene Blue Reduction Test
- Direct Microscopic Count

Experiment No from Book: **3-33**

## Samples needed for this experiment

- **Raw milk without any heat treatment**
- **Pasteurized milk**
- **UHT milk**

# Purpose and Importance

- Milk and Dairy Products are second most used food materials in food industry other than water.
- Foodborne pathogens associated with dairy products are;
  - *Listeria monocytogenes*
  - *Salmonella* Typhimurium
  - *Brucella miltensis*
  - *Bacillius cereus*
  - *Yersinia enterocolitica*
- Diseases and illnesses caused by these pathogens have severe effects which may lead to death.

# Coliform and other pathogens

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- Normally, milk obtained from a healthy animal should be free from any pathogens.
- But collecting materials and other contaminations cause pathogenic growth in milk and products.
- Heat treatment called pasteurization is the most common and effective method to remove these pathogens from raw milk.
- Utensils used for collecting milk may be contaminated with coliform in water.



# Tests and Analysis

- Methylene Blue Reduction Test
- Direct Microscopic Count
- Coliform Count
  - Media : VRBA
  - Method: Spread Plate from dilutions
  - Incubation: 37 C for 48 hours
  - Results: Count formed colonies and calculate # of coliforms / ml of milk sample

# Methylene Blue Reduction Test

## Purpose:

- This test indicates microbial quality of milk. It gives information about microbial load of raw milk.
- Main principle is Methylene Blue is  $H^+$  ion acceptor. When microorganisms in milk consume  $O_2$  they release  $H^+$ .
- $MB + H^+ \rightarrow LMBH$  (colorless)
- Time of decolorization of MB shows higher number of microorganisms.

# Methylene Blue Reduction Test

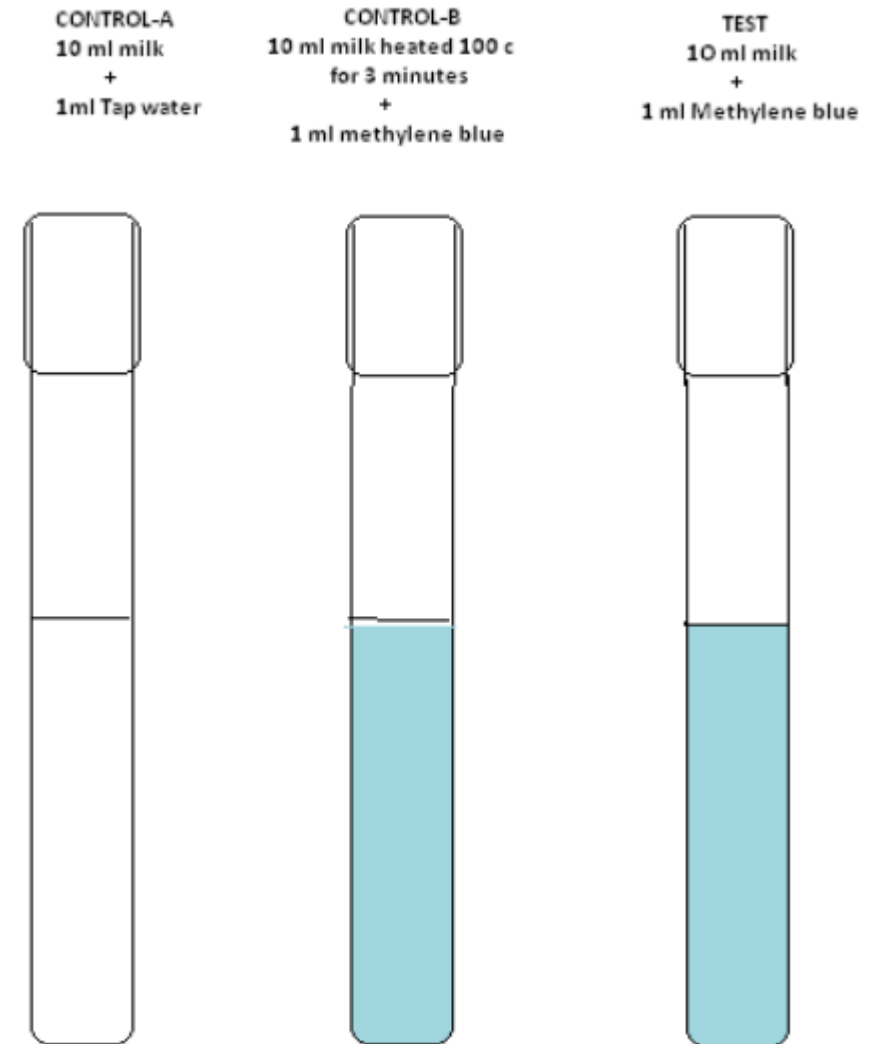
## Materials

- Milk sample (10 ml)
  - Methylene Blue Dye (10 drops)
  - Pasteur pipette for staining
  - Sterile pipette for sampling
  - Test tubes
  - Dark room
- Method
    - Basic dyeing with methylene blue and redox decolorization

# Methylene Blue Reduction Test

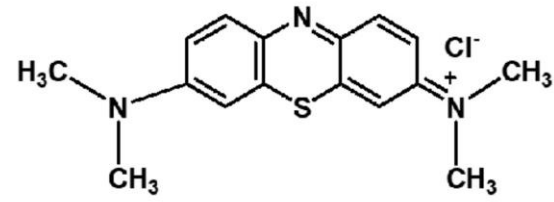
## Procedure

- Transfer 10 ml milk sample by using sterile pipette at aseptic conditions.
- Add 1ml Methylene Blue dye in each tube.
- Close the mouth of tubes and shake for mixing the milk and MB dye.
- Keep tubes at 37 C in a dark place and observe for decolorization of MB.

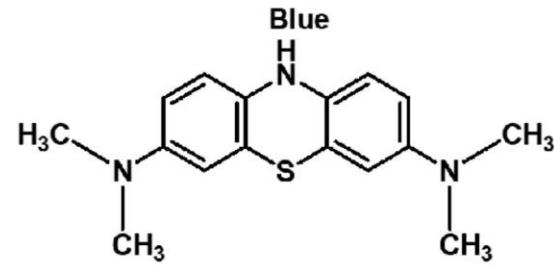




# Methylene Blue Reduction Test



Methylene Blue (MB)



Leucomethylene Blue (LMB)

Colorless



	TIME TAKEN FOR REDUCTION	GRADE
1	Reduction within 30 minutes	Poor quality
2	Reduction occurring between 1 to 2 hours	Fair
3	Reduction occurring between 3 to 4 hours	Good quality
4	More than 4 hours	Excellent quality

- Rapid Decolorization → High # of microorganisms
- Slow Decolorization → Low # of microorganisms
- Decolorization before 20 min means very bad quality raw milk.

# Direct Microscopic Count

- Principle

To determine the total count of bacteria in a sample of milk by Breed's Method.

Counting microorganisms stained with Methylene Blue under microscope at aseptic conditions and calculate # of microorganisms in ml of sample.

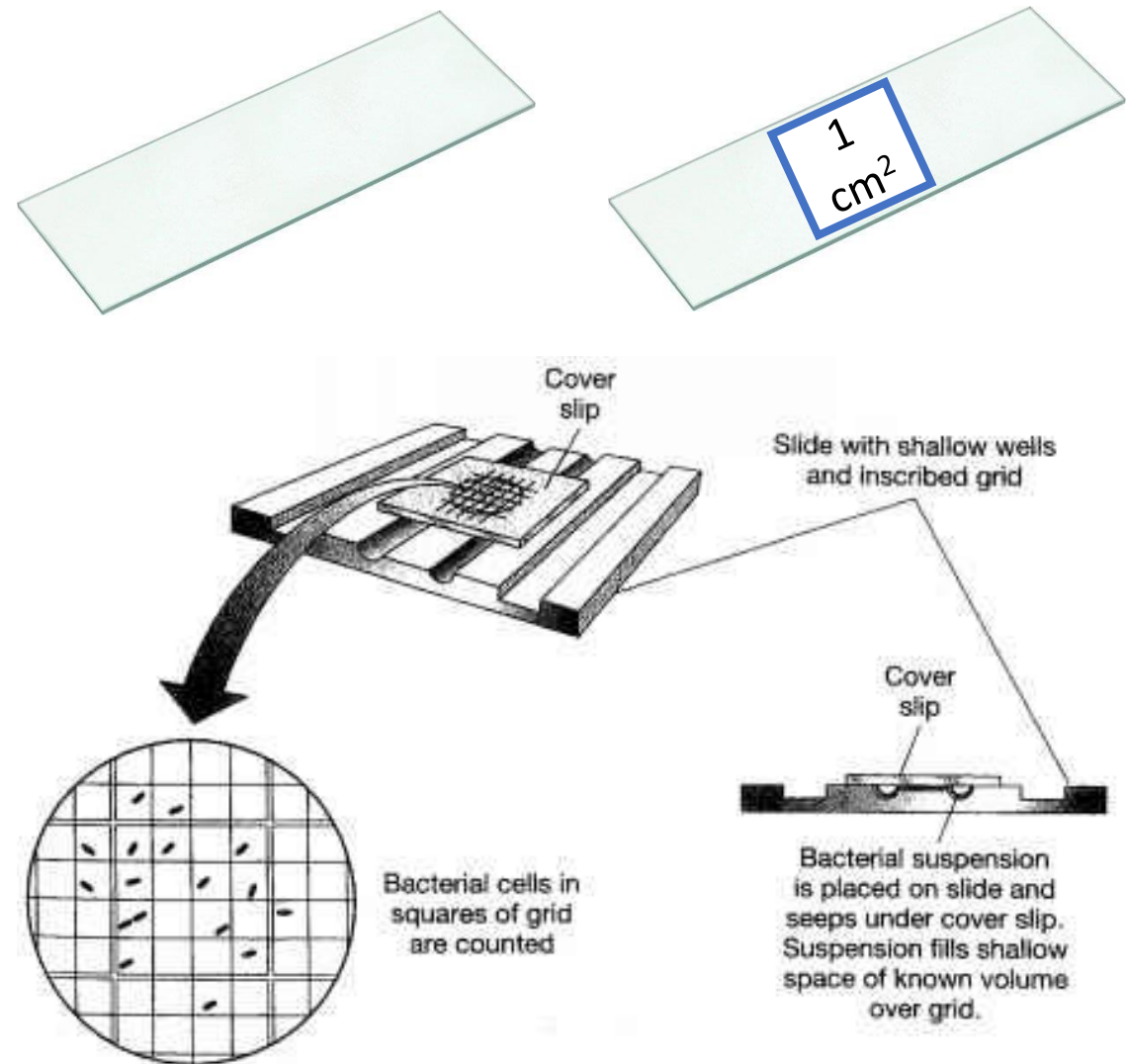
# Direct Microscopic Count

- Materials
  - Milk sample
  - Light microscope
  - Glass slide with grid lines
  - Pipette for sampling
  - Methylene blue for staining
- Method
  - Direct microscopic count

# Direct Microscopic Count

## Procedure

- Take a sterile glass slide
- Draw 1 cm<sup>2</sup> with marker pen.
- Take 0.01 ml of milk sample by using sterile pipette.
- Place this inoculum inside 1 cm<sup>2</sup> wait for air drying.
- Stain with Methylene Blue.
- Place a Cover slip and observe under 100X objective.
- Calculate # of bacteria in 1 ml of sample.



# Direct Microscopic Count

$\pi r^2$  = Area of field

where  $r$  = radius of field. Knowing the area of the microscopic field, it is then possible to determine the microscope factor (MF) by calculation:

(a) MF = Number of fields in 1 cm<sup>2</sup> (100 mm<sup>2</sup>)

Area of field in 1mm<sup>2</sup>

(b) Average number  $n$  of organisms or cells per field =

Number counted

Number of fields counted

(c) MF x  $n$  = Number of organisms or cells present in 1 cm<sup>2</sup>

(d) Since 0.01 ml of sample was spread over 1 cm<sup>2</sup>,

MF x  $n$  X 100 = Number of cells or organisms present in 1 ml of sample.

# Coliform Count

## Principle:

- Normally source of coliforms is dirty water. But coliforms may present in milk due to contamination through utensils and equipments used for collecting milk.
- Media: VRBA
- Method: Spread Plate
- Incubation: 37 C 48 hours.
- Count formed colonies on VRBA and calculate.

# Coliform Count

## Materials

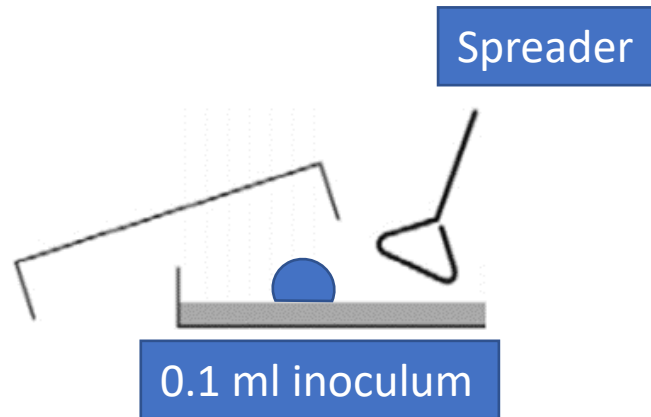
- Serial Dilution
- Milk sample
- Spreader
- Alcohol
- VRBA
- Sterile Pipette

## Method

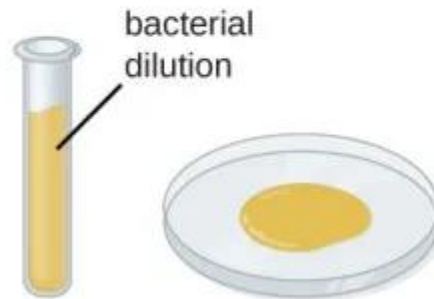
- Spread Plate method.

# Coliform Count

## Procedure:



1 Sample (0.1 mL) poured onto solid medium



### Spread Plate Method

2 Spread sample evenly over the surface



3 Plate incubated until bacterial colonies grow on the surface of the medium



- Incubate at 37° C for 48 hours.
- Count formed colonies and calculate # of coliforms / ml of milk



# Coliform Count

- # of Coliforms / g or ml of milk =  $\frac{\text{Count of microorganisms in one petri inoculum amount in one petri}}{\text{inoculum amount in one petri}} \times \text{Dilution factor}$

- Ex: Take only counts between  $30 < x < 300$  colonies into calculation.

- Non: TNTC

- -1: 546 → No calculation above 300

• -2: 186	→	$\frac{186}{0.1 \text{ ml}} \times \frac{1}{10^{-2}}$	→	$\frac{34}{0.1 \text{ ml}} \times \frac{1}{10^{-3}}$
• -3: 34	→			
• -4: 0	→	No calculation below 30		

- Take average of these two results

- # of microorganisms / g or ml of water =  $\frac{186000 + 340000}{2} = 263000$  microorganism / ml of water

# Evaluation of Results

- **Methylene Blue Reduction Test** → Indicates initial quality of raw milk.
  
- **Direct Microscopic Count**
  - Count microorganisms stained with dye and calculate Total Count
  
- **Coliform Count**
  - Presence of coliforms after calculation shows unsanitary conditions are present in utensils and equipments used for collecting milk.