Experiment 1: Assessing the Bacterial Load of Milk with Methylene Blue

Processed milk is pasteurized, that is, heated to a specific temperature for a specified amount of time, then cooled. This process reduces the amount of microorganisms present to non-disease causing levels. However, pasteurization does not kill all microorganisms in milk. The off-putting odor of expired milk is due to the growth and metabolism of bacteria. In this experiment, you will qualitatively assess the presence of bacteria in milk using the methylene blue reductase test. Methylene blue is a dye that is normally blue but turns colorless when it acted upon by bacteria (it is reduced, or it gains electrons, through the aerobic electron transport system). Methylene blue is essentially an oxygen sensor: the blue color disappears when the oxygen in the system is used. Aerobic bacteria metabolize oxygen; therefore, the faster the color changes, the more bacteria are present. This is a time course experiment, meaning that you will set up samples at different times then perform the assay on all samples at once. Please read the Procedure section carefully before beginning the experiment.

Materials:

- 4 Sterile screw-top tubes
- 8 Sterile, disposable transfer pipettes
- 0.05% Methylene blue solution
- Stopwatch
- Permanent marker
- 10 mL Graduated cylinder
- *60 mL Fresh refrigerated milk (whole, 2%, 1%, or fat-free)

*You must provide

Procedure

Use a permanent marker to label one of the tubes as “4 Hour Sample”.

Add 10 mL fresh refrigerated milk to the tube with a sterile transfer pipette. Place this tube at room temperature but out of direct sunlight.