Dye reduction test

- This method is indirect method to test the quality of milk.
- It serves to measure physiological and metabolic activity of bacterial population in raw milk or pasteurized milk.
- Oxidation-Reduction Potential of milk in udder (the mammary gland) of animals is low and it is about **0.23-0.25 volts.**
- During collection and transporting of milk samples, oxidation-reduction potential is increased and equal to **0.3** because oxygen enters it.
- If the milk contains high number of microorganism, will lowered oxidation-reduction potential to **0.01-0.06 volts** due to the consumption of dissolved oxygen by the bacteria
- The fact that dyes such as methylene blue loses its color (becomes reduced) in these environment is the basis for the dye reduction test.
- Milk of **poor quality** and **high number** of bacterial population will change the color of the dye quickly while milk with **good quality** and **low number** of bacterial population need several hours or more for color changing.

Advantages:

1. Raw milk will contain primarily ***Streptococcus lactis*** and ***Escherichia coli***, which are **strong reducers**; thus, this test is suitable for screening raw milk at receiving stations.
2. Simple for its performance.

➢ **Disadvantage:**

- The validity of this test is based on the assumption that all bacteria in milk lower the oxidation-reduction potential at 35-37°C, but large numbers of **psychrophilic, thermophilic** and **thermodurics** which do not grow at this temperature, would not produce a positive test.

Methylene blue reduction test

- The methylene blue reduction test is based on the fact that the color brought to milk by the addition of a dye such as methylene blue will disappear more or less quickly.
- The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism cause the color to disappear.
- The agencies responsible for the oxygen consumption are the bacteria. Though certain species of bacteria have considerably more influence than others.
- It is generally assumed that the greater the number of bacteria in milk, the quicker will the oxygen be consumed, and in turn the sooner will the color disappear.
- Thus, the time of reduction is taken as a measure of the number of organisms in milk although actually it is likely that it is more truly a measure of the total metabolic reactions proceeding at the cell surface of the bacteria.

Principle

- This test is based on if viable bacteria are present in the milk, they will reduce the methylene blue dye and decolorized the sample if kept for sometime in a dark place.
- The methylene blue is reduced due to depletion of oxygen in the milk as bacteria consume it.

Factors Affecting the Test:

1. Cold milk holds more oxygen than warm milk.
2. Pouring milk back and forth from one container to another increases the amount.
3. The kind of organisms affects the rate of reduction.
4. Light hastens reduction and therefore the test tubes should be kept covered.
5. The concentration of the dye should be uniform as an increased concentration lengthens the time of reduction.

Apparatus:

1. Screw-cap tubes or tubes with rubber stopper.
2. Raw milk sample of high and low quality
3. Water bath at 35 ◦C.
4. Methylene blue dye (o.o4 %).
5. Pipettes.

Procedure:

1. Label two screw-cap tubes with high and low quality of milk sample.
2. Using 10-ml pipette, transfer 10 ml of high quality milk to one screw-cap tube and with another pipette, 10 ml of low quality milk to the another tube.
3. Add 1 ml of methylene blue dye (o.o4 %) to each tube.
4. Cap tightly and invert the tubes several times.
5. Place the tubes in a test-tube rack and place the rack in the 35 °C- 37 °C water bath. After 5 minute incubation, remove the tubes from the water bath and invert several times to mix again.
6. Observe the tubes at 30-minute intervals for 8 hours for checking the discoloration.
7. Reduction is demonstrated by a change in color of the milk sample from blue to white.
8. Record your results

Interpretation

The speed at which the reduction occurs and the blue color disappears indicates the quality of milk as follows:

- If the viable bacteria decolorized the milk within 30 minutes, then the test is **positive** and milk is of **poor quality.**
- If the milk is not decolorized within 30 minutes, then the test is **negative** or milk is of **good quality**

- **The level of quality can be divided in to four types as fallow:**
- **Class (1): Excellent,** not decolorized at **(8) hours.**
- **Class (2): Good,** decolorized between **(6 – 8) hours.**
- **Class (3): Fair,** decolorized between **(2 – 6) hours.**
- **Class (4): Poor,** decolorized between **(30 min – 2 hours).**

The Resazurin Test:

- The resazurin test is conducted similar to the methylene blue reduction test with the judgment of quality based either on the color produced after a stated period of incubation or on the time required to reduce the dye to a given end-point.
- **Procedure:**

1. Prepare resazurin solution.
2. Place one ml of dye solution in a sterile test tube, and then add 10 ml of sample.
3. Stopper the tube, place in the incubator and, when the temperature reaches 37 ^{0}C, invert to mix the milk and dye.
4. Incubate at 37 ^{0}C.

- **Interpretation**
- Tubes are examined and classified at the end of **an hour** in the "one-hour test" or at the end of three successive hourly intervals in the "triple-reading test."
- The following relationships of color and quality are generally accepted:

Quality of Milk:	**Color of Sample:**
Class 1: Excellent	Not change (Blue).
Class 2: Good	Blue to deep mauve.
Class 3: Fair	Deep mauve to deep pink.
Class 4: Poor	Deep pink to whitish pink.
Class 5: Bad	White