**Microorganisms in milk**

In addition to nutritional value of the milk to human beings it is also considered as a typical media for the growth of many M.Os because it is rich in:

* Important proteins , carbohydrates , lipids , minerals & vitamins.
* Its optimal PH (6.7) & optimal moisture for microbial activity.

**Sources of milk contamination:**

* The milk contaminates by microbes during & after milking from (**external contaminations**) :

Atmosphere of the farm , soil , water, air , cattle feces, insects ,files .

* From mechanical milking ( milking machine , milk containers ,milk handlers . so should be all the tools , instruments & tubes clearing & sterilization .
* **Internal contaminations** :

Several microbes can be transmitted to milk from animal itself such as *bacilli.*

**Milk categories:**

1- **Raw milk :** this is from healthy animal contain low number of bacteria, between **102-103** bacteria/ml

The required bacterial number that is does not cause the changes in color & taste .

Raw milk spoilage

**Type of spoilage causative agent**

Coagulation *bacillus cereus*

Gas production (frothiness) *clostridium*

Viscosity in milk *alcaligenes*

Undesirable taste *Ps.fluorescence*

Red color in milk *serratia marcescens*

**2- thermized milk :** this a raw milk that has been heated at 56 C**o** for 15 seconds.

**3-pasteurized milk:** the milk must be exposed to 73 C**o** for 15 sec or 63 C**o** for 30 min , to control the on spoilage , pathogenic bacteria & to prolong the storage period.

**4-Boiling milk:** Boil the milk to 100 C**o** for 5 mins.

**5- sterilized milk:** by using high temp. 121 C**o** for 15-20 min.

Packed in a glass bottle , paper-based , or metal bottle, in this manner all microbes will be killed.

**6- dried milk(powder) :**

Made by the removal of water in milk with homogenization process & heat treatment to prevent spoilage.

If the examination of after treatment expose microbial growth this mean:

* The milk contamination by thermophilic bacterial spores.
* Or the milk insufficient heat treatment.
* Or the contamination happed because wrong procedure steps.

**Lab work**

**Dye Reduction tests**

The dyes most widely used for milk testing, Usually 1 ml of dye solution & 10 ml of milk are mixed in sterile-rubber stopper tubes & incubated at 37 C**O**.

 **1-Methylene blue reduction test (MBRT)**

This test is old but rapid & expensive. it indicates high or poor quality of milk .

**Principle :**

It depends on the reduction & decolonization of the dye by the metabolic activity of bacteria in milk & consumption of O**2** .the rate of reduction gives a measure of the degree of bacterial contamination.

**Procedure:**

1-carefully , pour 10 ml of the milk into test tube.

2-add I ml of the methylene blue solution ,the tube is closed & inverted & placed in a water bath at 37 C**O**.

3- two control tubes should be done with each test tube

 a- 10 ml of milk + 1 ml methylene blue solution heated in water bath to 37 C**O** for 30 min.

b-10 ml of milk + 1ml tap water.

After incubation, compare the test mixture with controls.

**Reading result:**

-complete de-colorization is +ve result with or without blue ring.

-colored milk with blue color is –ve result.

**Note:**

In untreated milk, The less time of the reduction of dye that mean increased contamination degree.

**2-** **The Resazurin Test**:

The reduction of resazurin takes place in two stages, first into blue and mauve then into the colorless.

Add 1 ml of resazorine + 10 ml of milk examine after 10 min

 Blue pink colorless.

**3-phosphatase test :**

This test is performed on pasteurized milk to determine if pasteurization has been successful or not.

**Principle:**

Test depends on the detection of phosphatase enzyme which is always present normally in raw milk. The enzyme is destroyed by the temperature in pasteurization .

If the phsophatase is not detected in the milk that mean the milk successfully pasteurized . if detected that mean insufficient heating or the raw milk is contamination .

In this test, add buffer substrate (nitrophenyl phosphate) which is colorless is hydrolyzed but by milk phosphatase convert to nitrophenol that is yellow .

**Turbidity test**

It is used to distinguish sterilized milk from untreated or sterilization process is effective or not.

**Principle:**

Milk that has been heated to 100 C**o** has had all the heat-coagulable protein denatured . if the milk not been exposed to boiled, the protein not denatured .

Addition the ammonium sulphate will be detected the coagulation & turbidity of the its protein .

**Procedure:**

1- four gm ammonium sulphate are added to flask.

2- 20 ml of the milk are added ,shaken for 1 min & left to 5 min.

3- the mixture is filtrated by Whatman filter paper.

4- examine for turbidity.

**Result:**

Absence of turbidity indicates that the milk has been heated to at least 100 C**O**, the milk exposed to satisfactory sterilized.

**Tests**

**1-Raw milk**

 Remember when we expect Highly contamination in the sample used No. dilution 10**-x .**

But when expect the sample has low contamination used No. dilution 10**-2** or 10**-3** .

Range of dilution 10**-1**-----🡪 10**-5**

**First step:**

**Used nutrient agar for general growth (aerobic plate count):**

 10**-5** 1ml or 0.01 37C**O** /24 hr-48hr.

**Second step:**

**used Selective media for bacteria that expected it contaminate of the sample**.

For the detection of coliform bacteria used:

**EMB**

**or Mac.**

 Coliform 10**-5** 1ml or 0.01 37C**O** /24 hr-48hr.

For the detection of spore former used:

**10-2**

**N.A**

 **Water bath 80 Co / 15 min cool 1ml 37Co /24hr**

**By pour method(1ml) or spread method(0.01)**

**Incubated the plates at 37Co/ 24hr-48hr**

**In an inverted position**

**Pasteurized milk boiling milk sterilized milk**

**D:(10-1,10-2,.10-3) D:(10-1,10-2,10-3) (10-1)**

**N.A**

**Third step:**

**Preparation slide from colonies that appeared.**