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.

Milk categories:

1- **Raw milk:** this is from healthy animal contain low number of bacteria, between 10^2-10^3 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 raw milk that has been heated at 56 C° for 15 seconds.

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

4-Boiling milk: Boil the milk to 100 C° for 5 min.

5-Sterilized milk: by using high temp. 121 C° 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 expose microbial growth after treatment this means:

- 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°.

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:

One raw milk sample and one pasteurized milk sample stored at room temperature for 48 h.

1-carefully, transfer 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°.

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

a- 10 ml of milk + 1 ml methylene blue solution $\longrightarrow$ 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 control.

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 means increased contamination degree.

This determination is made as follows:

- Reduction within 30 min indicative of very poor quality.
- Reduction occurring between 30 min and 2 hours indicative of poor quality.
- Reduction occurring between (3 and 6) hours are indicative of fair quality.
- Reduction occurring between (6 and 8) hours are indicative of good quality.

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 $\longrightarrow$ examine after 10 min

$\longrightarrow$ Blue $\longrightarrow$ pink $\longrightarrow$ 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° 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 protein.

Procedure:
1- four gm ammonium sulphate is 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.
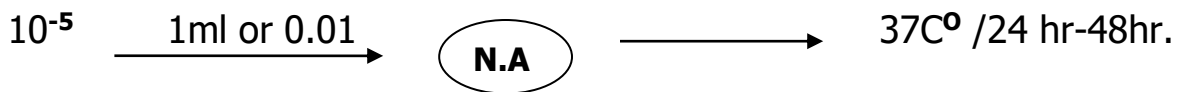
Tests

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}.

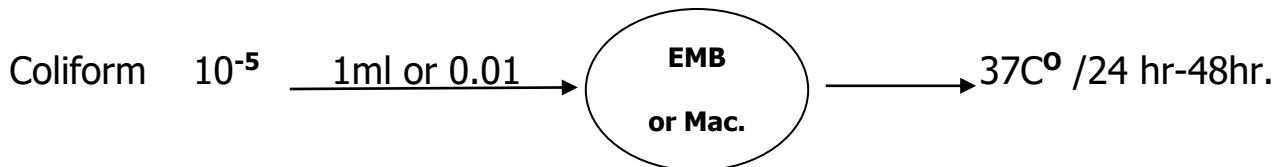Range of dilution 10^{-1}-----→ 10^{-5}

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

10^{-5} $\xrightarrow{\text{1ml or 0.01}}$ (**N.A**) $\longrightarrow$ 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:

Coliform 10^{-5} $\xrightarrow{\text{1ml or 0.01}}$ (**EMB or Mac.**) $\longrightarrow$ 37C^o /24 hr-48hr.

*EMB= Eosin Methylene Blue

*Mac= MacConkey agar

For the detection of spore former used:

10^{-2} → Water bath 80 C° / 15 min → cool → 1ml → N.A → 37C° /24hr
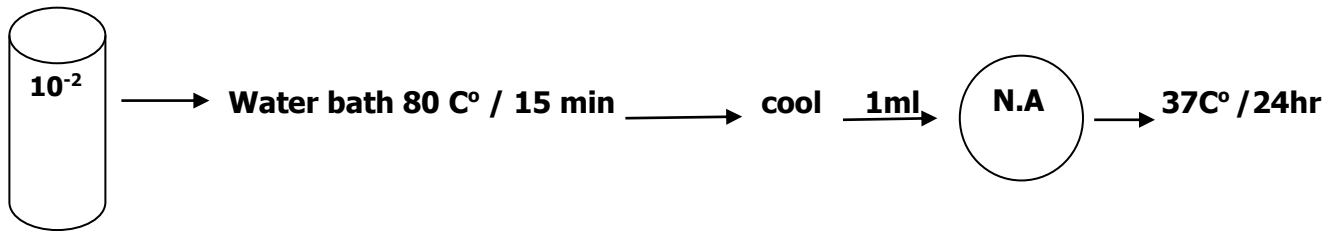
By pour method (1ml) or spread method (0.01)

Incubated the plates at 37C°/ 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	N.A	N.A
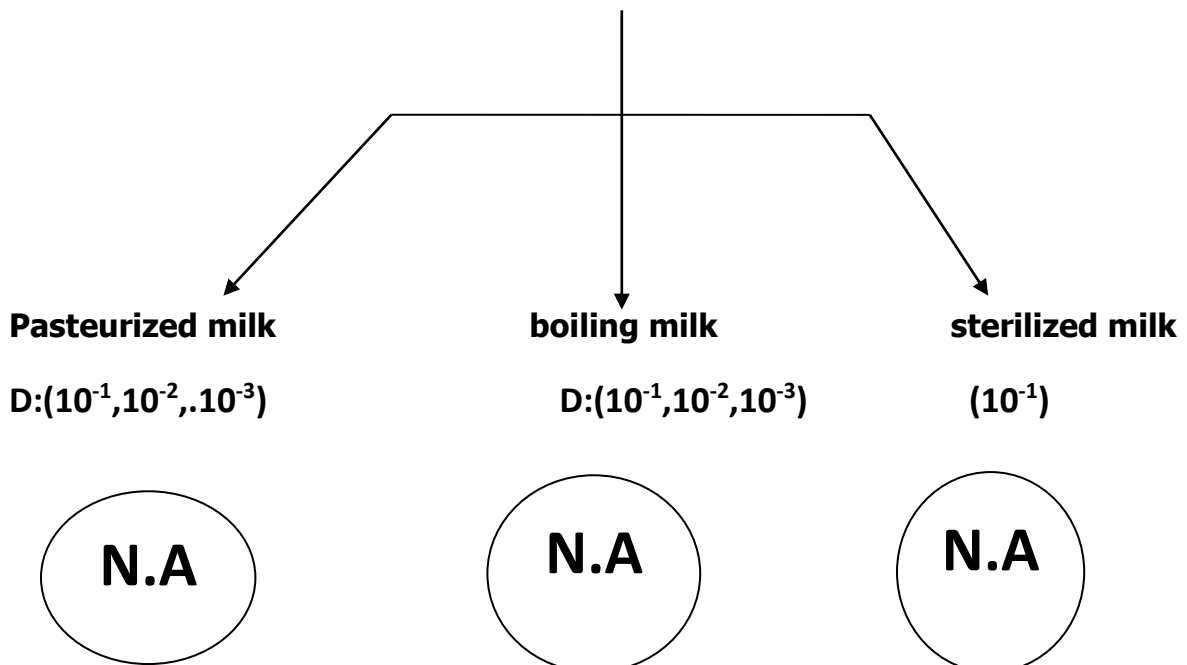
Third step:

Preparation slide from colonies that appeared.