Physical Properties of Milk

- Density
- Viscosity
- Freezing Point
- Acid-base Equilibria
- Optical Properties

Density

The density of milk and milk products is used for the following;

- to convert volume into mass and vice versa
- to estimate the solids content
- to calculate other physical properties (e.g. kinematic viscosity)

Density, the mass of a certain quantity of material divided by its volume, is dependant on the following:

- temperature at the time of measurement
- temperature history of the material
- composition of the material (especially the fat content)
- inclusion of air (a complication with more viscous products)

Viscosity

The viscosity of these products depends on the following:

- Temperature:
  - cooler temperatures increase viscosity due to the increased voluminosity of casein micelles
  - temperatures above 65° C increase viscosity due to the denaturation of whey proteins
- pH: an increase or decrease in pH of milk also causes an increase in casein micelle voluminosity.

Acid-base equilibria

Both titratable acidity and pH are used to measure milk acidity. The pH of milk at 25° C normally varies within a relatively narrow range

of **6.5** to **6.7**. The normal range for titratable acidity of herd milks is **13 to 20 mmol/L**.

Screening Tests:-

For diagnosis of diseased animals some of the tests are explained below.

(1). Surf Field Mastitis Test (SFMT):-

In SFMT **1ml** of **3% Surf solution (3 gm surf dissolved in 100 ml of distilled water)** is addedto **1ml of milk.** In positive Mastitis case there will be gel formation occur and severity of mastitis is depend upon the **consistency** of gel.

(2). Bromocresol Purple Test:-

This test is applied for the detection of Mastitis based on alteration of pH of milk.**2-3 drops of 0.9%Bromocresol Purple Solution is added to 3ml of milk.** Normal milk following addition of solution will appear as **yellow** while Mastitis milk will appear as **blue** or **purple**.

(3). White side Test:-

This test depends upon the increased Leukocytes content of milk. **5 drops of milk** is placed on glass plate underside painted black. Then **2 drops of 4% NaOH** is added to it. It is than rapidly stirred with broomstick for 20-25 seconds. In acute Positive case the mixture become thick and viscid, in chronic case white flakes are noted.

(4). Hotis Test:-

This test helps to detect presence of ***Streptococcus agalactiae.*** *Streptococcus agalactiae*ferment lactose of milk rendering it acidic. Thus, the indicator Bromocresol Purple turns to yellow.**0.5ml of 0.5% aqueous**

Bromocresol Purple solution is mixed to 9.5ml of milk in sterile test tube. This is mixed thoroughly and incubated at 37®C for 24 hours. *Streptococcus agalactiae*, if present in the milk will produce canary yellow colonies along the side of test tube.

(5). California Mastitis Test (CMT) .

Test Reagent:

Sodium Hydroxide - 1.5g

Teepol (Shell Chemical) - 0.5ml

Bromothymol Blue - 0.01g

Distilled Water - 100ml

This test is based on increased leukocytes count and increased alkalinity of the milk sample. This alteration is due to inflammatory exudates (leukocytes) and increased content of basic salt (alkalinity).

Equipment

Milk samples from each quarter are collected in a clean CMT Paddle. The CMT paddle has four shallow cups marked A, B, C, and D to help identify the individual quarter from which the milk was obtained. The CMT solution should be reconstituted according to package instructions.

How to use CMT

Step 1:

Take about 1 teaspoon (2 cc) milk from each quarter. This is the amount of milk that would be left in the cups if the CMT Paddle were held nearly vertical

Step 2:

Add an equal amount of CMT solution to each cup in the paddle.

Step 3:

Rotate the CMT Paddle in a circular motion to thoroughly mix the contents. Do not mix more than 10 seconds.

Step 4:

Read the test quickly. Visible reaction disintegrates after about 20 seconds. The reaction is scored visually. The more gel formation, the higher the score.

Reading the CMT

N = Negative

No infections. No thickening of the mixture.100,000 SCC

T = Trace

Possible infections. Slight thickening of the mixture. Trace reaction seems to disappear with continued rotation of the paddle. 300,000 SCC

Example: If all four quarters read trace there is no infection.

If one or two quarters read trace, infections are possible.

1 = Weak Positive

Infected. Distinct thickening of the mixture, but no tendency to form a gel. If CMT paddle is rotated more than 20 seconds, thickening may disappear. 900,000 SCC

2 = Distinct Positive

Infected. Immediate thickening of the mixture, with a slight gel formation As mixture is swirled, it moves toward the center of the cup, exposing the bottom of the outer edge. When motion stops, mixture levels out and covers bottom of the cup. 2.7 million SCC.

3 = Strong Positive

Infected. Gel is formed and surface of the mixture becomes elevated (like a fried egg). Central peak remains projected even after the CMT paddle rotation is stopped. 8.1 million SCC.

Any reaction of T (trace) or higher indicates that the quarter has subclinical mastitis

CMT	Score Somatic Cell Range	Interpretation
N (Negative)	0 – 200,000	Healthy Quarter
T(Trace)	200,000 – 400,000	Subclinical Mastitis
1	400,000-1,200,000	Subclinical mastitis
2	1,200,000-5,000,000	Serious Mastitis Infection
2	Over 5,000,000	Serious Mastitis Infection

(6). Somatic Cell Count:-

Somatic cells are the epithelial cells and leukocytes coming down in the milk during udder infections. Somatic Cell Count **(SCC)** of the milk of healthy cow is 200,000 / ml and 100,000 increases in the cells (300.000 cells / ml) means that the milk is from mastatic cow. Health impact varies much on Somatic Cell Count, as increase of 100,000 SCC / ml in the milk, leads to SCC value from 400,000 to 500,000 that results in to 25 % less cheese production.

Importance of Somatic Cell Count:-

Somatic Cell Count (SCC) provides three important functions:

1. Monitoring of prevalence of Mastitis in dairy cow.
2. Act as indicator of raw milk in all processes.
3. Act as indicator of hygienic condition of milk at a dairy farm.

Standard Somatic Cell Count in Milk (SCC / ml) for Different Countries Country SCC / ml

United States	750,000
Australia	400,000
Canada	500,000

Procedure of determination SCC / ml of milk:-

1. Take a clean slide and divide it in to two Squares of 1cm2 areas with the help of diamond pencil.
2. Put 10µl (0.01ml) of milk on each area and allow for air dry.
3. Then put the slide in Xylene for 2-3 minutes for defatting.
4. After defatting allow the slide for air drying again and then fixation of smear by 95% ethanol for 5 minutes. After fixation again allow slide for air drying and then stain by 10 % Giemsa solution for 30 minutes.
5. Now wash with tap water and observe under oil immersion (100X) lens of microscope and counting of leukocytes is done.
6. Observe 10 fields on a square and count number of cells in each field. Then add all the number of cells obtained from 10 fields and divide by 10 to get average number of cell in each field.
7. Than multiply the average number of cells with 5000 as 1cm2 area has 5000 fields. This is number of cells in 0.01ml of milk, to convert it into ml multiply the number of cell in 0.01 ml with 100, it will give number of cells per ml of milk (This can directly be obtained by multiplying the average no of cells with 500000).

8. 0-200,000 number of cells per ml of milk is considered normal and more than this is considered positive. More than 500, 0000 cells per ml of milk is taken as +++ mastitis .

Ex. Total leukocyte in 10 field = 18 , average no= 18/10 = 1.8

N0 × 5000 , 1.8× 5000 = 9000 cells / square tat is No of cells per 0.01 ml

For ml , the No of cells per 0.01 ml × 100

9000 × 100 = 900000 cells/ml

Results interpretations for Somatic Cell Count / ml of milk

SCC /ml of milk	% of quarter affected
300,000	6.2
400,000	12.8
750,000	24.3
1000,000	32.6

(7). Bromothymol Blue Test:-

The reaction of milk can be tested by this method. This test has been used successfully to diagnose mastitis. For this BTB card test paper may be prepared in the laboratory from Whatman filter paper No.1. The diagnostic card can be prepared by adding one drop of BTB test solution (1.6 gm bromothymol blue in 100 ml ethanol) at 4 different spots on the paper and indicated as left fore (LF), left hind (LH), right fore (RF) and right hind (RH). One drop of the suspected milk has to be put directly on the spot and the change of the color is to be noted, the change of color may be scored as “(pale green) i.e, normal quarter and “+” , “++” , “+++” (according to the change of color from moderate green to dark green). The only disadvantage this test is that cow in later lactation may give false positive reaction.

(8). Catalase Test:-

There is presence of catalase in every living cell. Leucocytes contain increased quantity of this enzyme. The determination of catalase will give fair indication of the presence of leukocytes. In udder infection the number of leukocytes is increased in the milk, Therefore, Catalase determination will indicate about the presence or absence of infection. The amount of catalase present is determined by the ability to break down the Hydrogen Peroxide (H2O2) to Oxygen and Water. For this test **1% H2O2** is used.

Reaction: 2H2O2 + Catalase = 2H2O + O2

(9). Chloride Test:-

This test demonstrates the presence of the increased quantity of chloride in mastitis milk. Normal chloride content of milk is 0.08-0.14 g. but in mastitis due presence of inflammatory exudates the chloride content is increased.

Test is as follows:

Solution A: Silver nitrate = 1.3415 gm

Distilled water = 1000ml.

The solution should be kept in amber color bottle.

Solution B: Potassium Chromate = 10 gm

Distilled water = 100ml.

Procedure:

1. Take 1 ml of milk in a test tube.
2. Add 5 ml of solution A.
3. Then add 2 drops of solution B to the mixture.
4. The mixture is to be mixed well by inverting the tube.

Interpretation:

A yellow color denotes more than 0.14 % chloride in the milk and a brownish red color indicates less than above amount.

The reaction may be deduced such as**: AgNO3 + Milk Chloride = AgCl-PPT**

The yellow color is due to Potassium Chromate. If the amount of chloride is 0.14% or less, all the Silver Nitrate will not be used. In this case reaction will take place with addition of Potassium Chromate.

AgNO3 + K2CrO4 = Ag2CrO4 (Brownish Red Silver Chromate)

The Brownish Red color indicates a **Negative result.**