**Laboratory Examinations Of Milk**

The biological functions of milk is to supply nutrition and immunological protection to the young mammal. In some species, milk is the only food consumed for weeks or months, Therefore, it must furnish all nutritive requirements for maintenance and growth, such as energy, amino acids, minerals, and vitamins.

**Several different** diagnostic tests were routinely used to evaluate milk quality in animals. These include:

1. Physical examination of the milk by strip-cup examination.

2. Chemical examination of the milk.

3. Indirect leukocyte count (screening tests).

4. Direct leukocyte count (microscopic examination).

5. Microbiological examination, to identify the organism by its reaction in the culture media as in Hotis test.

**Physical Examination of Milk**:

**a-Milk temperature :**

The temperature of milk samples can be determined at the collection point using the suitable thermometer or temperature sensor .

**b-Strip-cup test:**

This test is done by naked eyes which look for abnormalities in milk inside the cup, such as flakes, or clots of milk, fibrin, and mucous. Any of which denotes active infection of the udder. Latent infection rarely show these changes.

**c-Boiling test :**

Boil a small amount of milk in a spoon, test tube or other suitable container. If there is

clotting, coagulation or precipitation if the milk has failed the test. the milk must contain many acid producing microrganisms or the milk has an abnormal high percentage of proteins like colostral milk.

**d-Determine of Specific Gravity of Milk:**

***Objective:***

To determine the basic nature of milk to decide the nature of adulteration of milk.

***Procedure:***

Lactometer is used as an instrument for rapid determination of density of milk. The density is determined with a glass instrument called lactometer. It is based on the principle of floatation which displaces specific volume of milk on floatation.

**Normal milk S.G :** For cow : 1.028 - 1.032,For Buffaloes: 1.030 -1.034.



**Chemical Examination of milk :**

**Different tests for evaluation of milk ….**

**Determination of milk pH:**

Normal milk has a pH of 6.4 - 6.8 .

1. Milk from affected udder is abnormally alkaline, with degree of alkalinity depend upon the severity of inflammation, since abnormal milk have a pH as high as 7.4 .

2. pH should be determined on freshly drawn milk, although milk held at refrigerator temperatures for 24 - 48 hours may be used.

3. Although determination of milk pH has limited value in detecting the existence of udder inflammation.

several methods can be used, but the most

common of which is the use of indicators that change color at or near the normal pH.

5. Both bromthymol blue and bromcresol purple have been widely used for the detection of pH alterations in mastitic milk.

***Bromthymol blue test (BTB):***

This test will indicate alteration associated with most acute or subacute cases of mastitis, but in chronic conditions there may not be sufficient pH changes detected, because there is so little active inflammation that exudate is not produced in a quantity sufficient to cause a pH change.

***Procedure :***

1. 1 ml of bromthymol blue solution is pipetted into a 15 ml capacity test tube.

2. 5 ml of milk added with a pipette.

3. When bromthymol blue is added to normal milk, yellow color appear.

4. Alkaline milk show green to greenish-blue color when bromthymol blue

added, depending on the amount of alkalinity.

5. Increase the alkalinity is due to the presence of exudates containing unusually large amounts of alkaline salts derived from blood and lymph.

6. In late stage of lactation, the test may give false-positive reaction (milk at this stage being normally more alkaline than the other stage of lactation).

**Note:**

Bromthymol blue solution consists of (1 g) bromthymol blue, (160 ml) N/100 sodium hydroxide, (590 ml) distilled water.

***Bromcresol purple test (BCP):***It is used in the same manner as bromethymol blue for determination milk pH. It has the advantage of becoming yellow in a pH range below 5.2 and thus abnormally acid milk may be detected.

1. Add 0.5 ml of bromcresol purple solution to 9.5 ml of milk.

2. Normal milk produces pale grayish purple color.

3. Abnormal milk becomes deep purple with increased alkalinity.

**Note:**

3 Bromcresol purple solution consists of (0.9 g) bromcresol purple powder, (100 ml) distilled water.

***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.

***Procedure of chloride test:***

1. Add 5ml of silver nitrate solution to 1 ml of milk.

2. Add 2 drops of potassium chromate solution and mixing by inversion of the tube.

3. Read the result as follows:

 The appearance of yellow color indicates that the sample contains more than 0.14% chlorides .

 Brownish red color indicates that the sample contains less than 0.14% chloride

 Cow in either early or late lactation may give false positive reaction to the chloride test because of the normal physiological process of the udder.

***Whiteside Test:***

**Principles:**

This test based on that the nucleic acids of the leukocytes of milk form sodium salt with NaOH producing a gelatinous mass to which serum solids and fat globules become absorbed to produce a characteristic precipitate of the reaction.

***Procedure of th test:***

1. On clean glass slide add 5 drops of milk.

2. Add one drop of sodium hydroxide solution 5%, mix with loop or glass rod.

3. Read after 0.5 to 1 minute, white flakes or clumps indicate abnormal milk.

**Interpretation:**

 ***Negative:*** the mixture is milky and opaque and entirely free of precipitant. In such animals, the leukocyte count is generally

 under 500,000/ml.

 ***Trace:*** the mixture is opaque and milky, but fine particles of

coagulated material are present. In such milk, the total leuko-cyte count is usually between 500,000 and 1.5 million.

 the background is less opaque but still some what milky with larger particles of coagulated material being present and thickly scattered throughout the area. The leukocyte count is usually between 1 and 2 million.

 ***Positive 2+:*** the background is more watery and are large clumps of coagulated materials are present. The total leukocyte count is usually over 2 million.

 ***Positive 3+:*** the background is very watery and whey-like, with large masses of coagulated material forming into strings and shreds.

***Positive +1 Positive +3***

***California Mastitis Test (CMT):***

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).

**Principle of the test :**

Anionic surface active reagent and an indicator, bromcresol purple. The leukocytes of

milk (somatic cells) are ruptured by the reagent releasing their DNA, which is the

principle in the test.

***Procedure of California mastitis test:***

1. A white plastic paddle of spherical design, and having 4 shallow cups is used.

2. About 2 ml. of milk is needed from each quarter.

3. The schalm reagent is added in equal volume to the milk.

4. The reagent and milk are mixed by gentle circular movement of the paddle in

a horizontal plane.

5. The reaction is visible grossly, and with experience, the intensity of reaction,

can be graded in a manner that correlated well with the number of somatic

cells in the milk.

**Result:**

 ***Negative:*** the mixture remains liquid with no evidence of precipitate or

gel formation.

 ***Trace:*** slight precipitate which disappears with continuous movement.

 ***Positive 1+:*** distinct ppt., but no tendency to gel formation.

 ***Positive 2+:*** the mixture thickens immediately with a slight gel

formation. As the mixture is caused to swirl, it tend to move towards

the center. When the motion is stopped, the mixture covers the bottom

again.

 ***Positive 3+:*** distinct gel formation which tends to adhere to the bottom

of the paddle.

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***Hotis Test:***

This test helps to detect presence of *Streptococcus agalactiae* since *Streptococcus*

*agalactiae* ferment lactose of milk from infected quarter.

**Procedure :**

1. 9.5 ml. of suspected milk.

2. 0.5 ml. of 0.5% bromcresol purple solution.

3. Incubate for 24 . 48 hours at 37ºC .

**Result:**

 ***Light purple***, no change: Normal milk.

 ***Yellow colonies*** on the slide of the test tube or yellow sediment:

*Streptococcus agalactia* infection.

 ***Red or rusty flakes*** (agglutinated colonies) on the slide, or red

sediment: Presence of staphylococci or micrococi (72 hours

incubation).If more than one type of organisms is present, or when the

sample is contaminated,as combination of changes may obscure test

reaction .

**A B C D E**

 ***A, negative tests*: color remains unchanged, no flake formation**

 ***B, C, D,and E,* characteristic of the several changes typical for *Streptococcus agalactiae*,**

**acid formation and few to many yellow or brown flakes**

 **F G H I J**

 ***F, negative tests*: color remains unchanged, no flake formation**

 ***G,* purple column, white flakes (diphtheroides)**

 ***H, I,* slightly acid, rust-colored flakes (*Staphylococcus aureus*)**

 ***J,* slightly acid, yellow sediment, no flakes (nonhemolytic staphylococci and streptococci**

**other than *S. agalactiae***

***Surf Field Mastitis Test (SFMT):-***

In SFMT 1ml of 3% Surf solution (3 gm surf dissolved in 100 ml of distilled water) is

added to 1ml of milk. In positive Mastitis case there will be gel formation occur and

severity of mastitis is depend upon the consistency of gel.

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***Catalase Test:***

**Principles of the test:**

There is presence of catalase in every living cell, including leukocytes, contain

catalase, which is capable of decomposing hydrogen peroxide to gaseous oxygen.

Catalase is not present in significant quantities in normal milk except in the early or

very late stages of lactation. A quantitive catalase determination reveals information

regarding to the number of leukocytes present in a milk sample.

***Procedure of catalase test:***

1. Mark 15 ml screw cap test tubes at a 10 ml volume.

2. Add 1 ml of freshly prepared 3% hydrogen peroxide and 9 ml of milk to the

mark, the hydrogen peroxide must be fresh.

3. Fill the test tube with water.

4. Cap the tube loosely and invert it in a test tube rack placed in a shallow pan.

5. Incubate for 3 hours at room temperature.

6. Measure the length of the gas column following the incubation period.

7. The percentage of oxygen is calculated by the following formula:

**(Length of gas column × 100 / length of milk column)**

8. 40% of catalase in milk should be considered abnormal.

9. 30 . 40 % of catalase in milk should be considered suspect.

***Somatic Cell Count:-* (SCC)**

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.

**Procedure of determination SCC / ml of milk:-**

1. Take a clean slide and divide it in to two Squares of 1cm 2 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.

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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 1cm 2 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.

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