

STORAGE

The examination of milk should be performed immediately after its collection. But if delay is expected in examination, it should be kept in refrigerator at 4°C.

Physical Examination

1. *Colour*: Normally the colour of milk is white or creamy white but in acute mastitis it may become reddish due to presence of blood. In chronic mastitis or subacute mastitis it may become watery. The colour of milk becomes yellowish during colostrum period. The pink to brown colour of milk is seen due to phenothiazine treatment.
2. *Specific gravity*: The specific gravity of normal milk is 1.015 to 1.037 which may get altered due to mastitis.
3. *Reaction*: The pH of normal milk is 6.4 to 6.6. But in mastitis it becomes alkaline upto 7.4 due to presence of sodium bicarbonate in the milk. The reaction can be determined by using pH strips.
4. *Odour*: Normally the odour of milk is pleasant but it may turn obnoxious due to mastitis caused by *Actinomyces pyogenes*. In ketosis the odour of milk becomes sweet, and fruity.
5. *Consistency*: The colostrum or first milk of the animal just after parturition is viscous. The watery consistency occurs due to poor feeding and chronic mastitis. During mastitis (acute and subacute), the milk contains fine as well as large flakes. The consistency of milk is determined by using strip cups even under field conditions.
6. *Strip cup test*: This test is useful in field conditions for physical examination of milk. In this test, enamel plate divided in four strip cups is used. The bottom of the plate is black coloured so that it gives a good contrast to easily observe the milk flakes. The comparison of milk from all 4 quarters can be carried out simultaneously. The milk flakes can be seen by tilting the cups at an angle. This test is very useful in primary screening of animals for mastitis.

Chemical Examination

1. Bromothymol blue test:

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.

- 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 BTB is added to normal milk, yellow color appear.
- 4- Alkaline milk show green to greenish-blue color when BTB 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).

2. 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.

3. Chloride test:

The presence of more than %0.14chloride content in milk is considered abnormal. For this test.

1. Take 5ml of silver nitrate solution in a test tube and add 2 drops of potassium chromate solution as indicator.
2. To this add 1 ml of milk sample and mix it properly.

3. If the chloride is less than %0.14 then it will remain brownish red while in positive case it will become yellow indicating more than 0.14% chloride content in milk.
4. However, this test may give false positive reaction during early and late lactation.

4. *White slide test:*

This test is based on the fact 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 .

1. In this test, 4-5 drops of test milk sample are placed on a clean dry glass slide.
2. Add one drop of sodium hydroxide solution 5%, mix with loop or glass rod.
3. If the milk is from an animal having mastitis, it becomes thickened and flakes appear.
4. While the negative milk sample remains the same.

5. *California mastitis test:*

This test is based on increased number of leukocytes and increased alkalinity in milk due to mastitis. The leukocytes of milk (somatic cells) are ruptured by the reagent releasing their DNA, which is the principle in the 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 California 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 .The results are read as follows:
 - a. Liquid milk with no streaks or precipitation: Negative for mastitis.
 - b. Streaky fluid: +
 - c. Slimy : ++
 - d. Gelatinous : +++

6. *Hotis test*:

1. Take 0.5 ml of 0.5% bromocresol purple and sterilize it by autoclaving.
2. Mix 9.5ml milk sample after cooling and incubate it at 37°C for 24 hours.
3. The appearance of yellow colonies along the sides of tubes or on bottom indicates the infection due to *Streptococcus agalactiae*.
4. Red or rusty flakes on the side, or red sediment: Presence of *staphylococci* or micrococi (72 hours incubation).

Cytological Examination

Leukocyte count:

1. Mix the milk sample thoroughly and take out 0.1 ml of mixed milk and place it on a clean, dry glass slide.
2. Mark the area on central portion of slide (1 cm) and spread the milk sample with bacteriological loop in this area.
3. Dry the smear and dissolve the fat by rinsing it in xylene for 2-5 minute.
4. Fix the smear with alcohol for 5minute and stain with methylene blue for 1 minute.
5. Remove the excess stain by immersing the slides in alcohol.
6. Count the leukocytes under oil immersion of the microscope.
7. The cells of 10 fields are counted and averaged and multiplied by 500,000 to get the total number of leukocytes per ml of milk.

Bacteriological Examination

1. Direct microscopic examination:

This is rapid procedure to know the type of infection and total number of organism present in the milk. In this method like leukocyte count,

1. Take 0.1 ml properly mixed milk sample and place on centre of a clean and dry glass slide.
2. Mark the 1 cm central area of slide and spread the milk sample by bacteriological loop.
3. Air dry the slide with gentle heat and fix it on flame.
4. Stain the smear with Newmann's stain for 2minute, wash the stain and air dry the smear.

5. Examine under oil immersion of the microscope.
6. Count the single bacteria or isolated clumps in any 10 fields and average them.
7. To get the bacterial count per ml, multiply the count with 500,000 .

2. Cultural examination:

The milk is collected in sterilized vials and stored in refrigerator. The milk sample is inoculated on bacteriological media.

The following media used :-

1- Edward media

This media inhibit staphylococcus and coliform bacteria are readily distinguished by their characteristic colonies.

2- Sodium azide crystal violet blood agar .

For detection of Streptococci and micrococci and Staphylococci and inhibit grow of all organism. Staph. colonies are large than Str. Colonies.

3- Salt agar

Sodium chloride added to this media 7.5 % for Staphylococci.

4- Glycine tellurite agar

For Staphylococci as black colonies where other colonies produce transparent colorless colonies .