

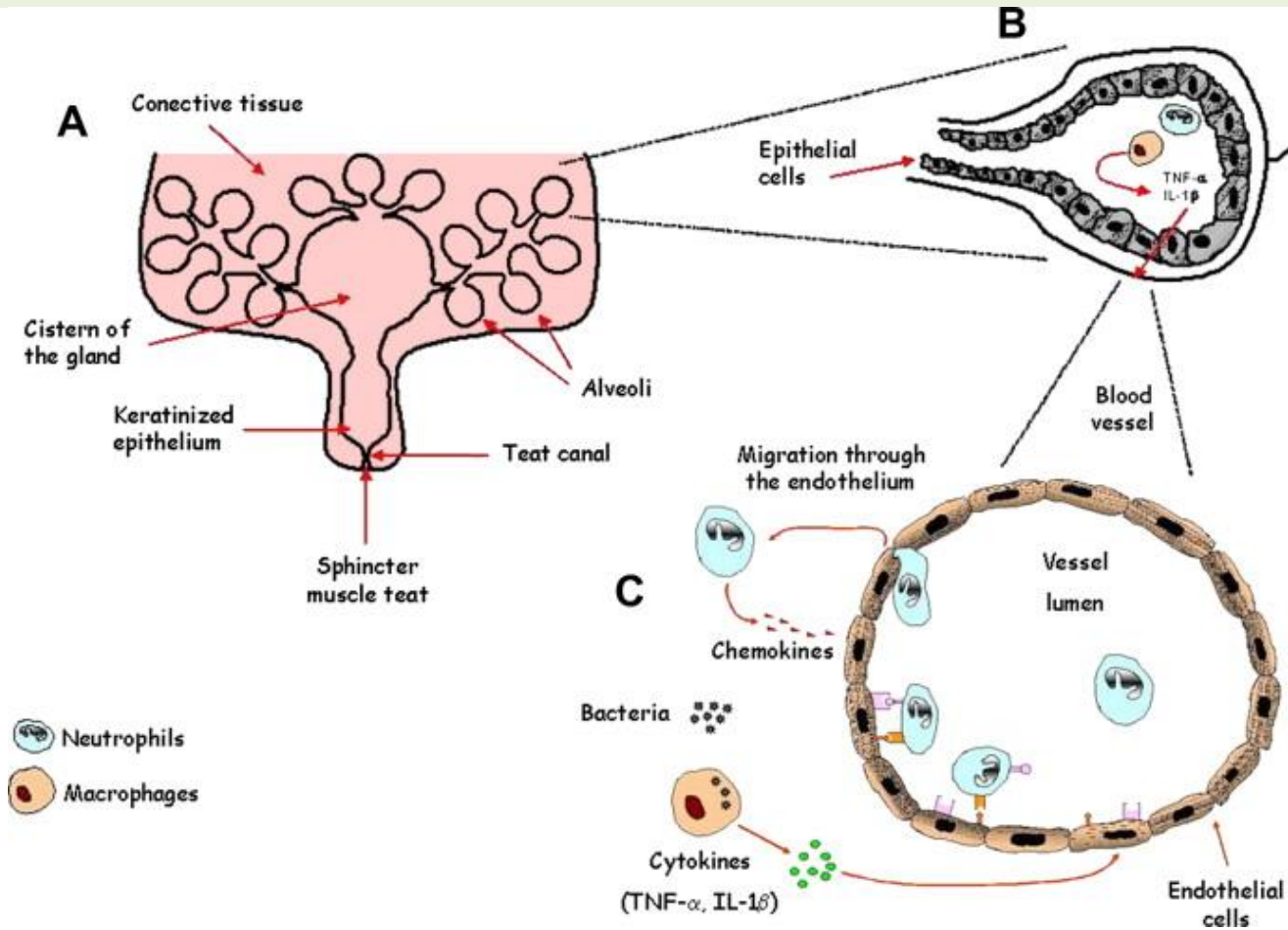


Examination of Milk (Physical and Chemical)

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Clinical Pathology
4th Class



Anatomy of Mammary Gland



Physical Examination of Milk

Physical examination of milk means the investigation about the alteration in milk Color, Odor and Consistency.

Color: Normally the color of milk is white or creamy white but the color may turn :

- **Reddish** due to presence of blood.
- **Watery** In chronic mastitis or subacute mastitis.
- **Yellowish** during colostral period, feeding of carrots, tetracycline therapy.

Odor: Normally the odor of milk is pleasant but it may turn :

- **Obnoxious** due to mastitis caused by *Antinomies pyogenes*.
- **Sweet and fruity** due to ketosis

Consistency: Change in milk consistency:

- **Viscous** due to colostrum.
- **watery** due to poor feeding and chronic mastitis.
- **Flakes** due to mastitis.

Physical Examination of Milk

Physical examination of Milk includes:

- 1. Boiling test**
- 2. Specific gravity**
- 3. Strip-cup test**

Boiling test

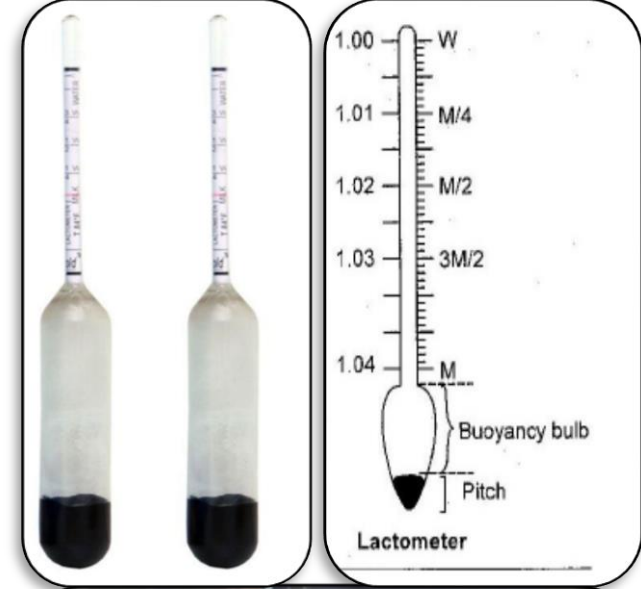
- Boil a small amount of milk in a spoon, test tube or other suitable container.
- If there is clotting, coagulation or precipitation it means that milk has failed the test.



This can be explained as milk contain many acid or rennet producing microorganisms or the milk has an abnormal high percentage of proteins like colostrum milk.

Specific gravity

- **Objective:** To determine the basic nature of milk to detect the 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.



Strip-cup test

- **Objective:** To detect clinically abnormal milk showing discoloration, flakes, clots.
- The clots and flakes observed in abnormal milk are somatic cells, fibrin, and other blood components that have entered the affected quarter to fight infection



Chemical Examination of Milk

Chemical examination of milk includes the estimation of:

- Milk PH
- Milk chloride
- Electrical conductivity
- White side test
- California mastitis test (CMT)
- Milk portions, Lactose, Fat, ions, minerals, vitamins and Enzymes

Milk pH

- The pH of normal milk is 6.4 to 6.8.
- The increase alkalinity of milk is greatly dependent on the presence of alkaline salts that leaks from the blood and lymph during exudate formation as a result of inflammation.

- Methods used for estimation milk pH includes:
 1. pH meter.



2. Impregnating absorbent filter paper with an indicator (This method is not accurate).
3. The most common dyes used are bromocresol purple (BCP) and bromothymol blue (BTB).



*** pH test may give false positive reaction in later stage of lactation.**

Bromothymol Blue (BTB).

**1ml (0.2% bromothymol blue solution) + 5ml
(freshly collected milk sample)**

**Normal milk
gives yellow
color**

**Alkaline milk gives
Green – Greenish
blue color**



5.5 6.0 6.5 7.0 7.5 8.0

Bromocresol Purple (BCP)

**0.5ml (Bromocresol Purple solution) +
9.5ml (freshly collected milk sample)**

**Normal milk gives
pale grayish
purple color**

**Alkaline milk gives
deep purple color**



5.0 5.5 6.0 6.5 7.0 7.5

Chloride test

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

5ml of (silver nitrate solution) + **1ml** (milk)+
2 drops (potassium chromate solution)

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

yellow color
indicates that the
sample contains
more than 0.14%
chlorides

Electrical conductivity

Draminski Mastitis Detector: A device used for detecting sub-clinical mastitis in its earliest, visually undetectable stage based on the differences in salt concentrations that occur between infected and uninfected quarters of the same cow.



Electrical conductivity

Presence of bacterial infection in the affected quarter causes

- **Increase** in the sodium, chloride (salt) ions and milk pH.
- **Decrease** in potassium ions and lactose

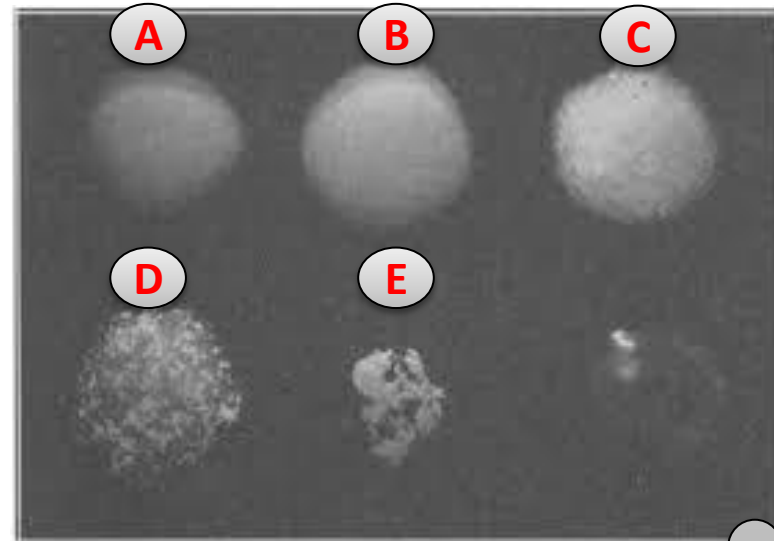
*Sodium and chloride are elevated in infected quarters because they leak from blood into milk during the process of inflammation.

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:

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.



Typical reactions obtained with Whiteside test.

A) negative, **B)** trace, **C)** 1 +, **D)** 2 +, **E)** 3 +.

Whiteside test 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 leukocyte count is usually between 500,000 and 1.5 million.
- **Positive 1+:** 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.

California mastitis test (CMT)

- CMT Solution consist of Anionic surface active reagent and an indicator, Bromocresol purple. The leukocytes of milk (somatic cells) are ruptured by the reagent releasing their DNA, which is the principle in the test.
- This test is based on increased leukocytes count and increased alkalinity of the milk (due to inflammatory exudates and leukocytes).



Procedure California Mastitis Test (CMT)

1. A white plastic paddle of spherical design, having 4 shallow cups is used
2. About 2 ml. of milk is needed from each quarter.
3. The CMT 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.

Interpretation of CMT Results

- **Negative:** the mixture remains liquid with no evidence of precipitate or gel formation.
- **Trace:** slight precipitate which disappears with continuous movement.
- **Positive 1+:** distinct precipitate, 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 tends to move towards the center leaving the edge of the cup exposed. 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 and during swirling a distinct central peak forms.

California Mastitis Test Scores
Correlation of CMT Score with Somatic Cell Count

CMT Score	Somatic Cell Range		
N	0	to	200,000
T	200,000	to	400,000
1	400,000	to	1,200,000
2	1,200,000	to	5,000,000
3	Over 5,000,000		

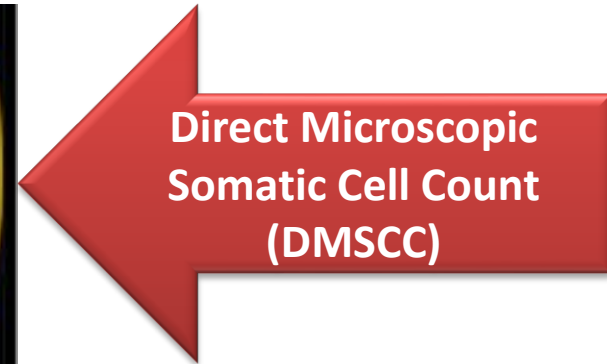
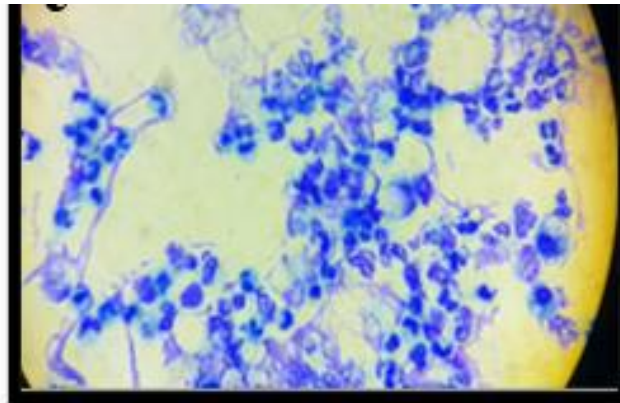
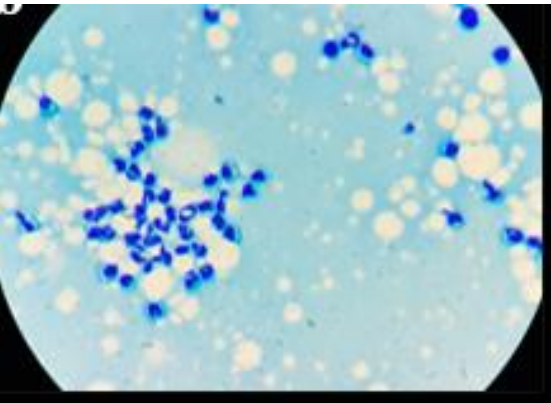
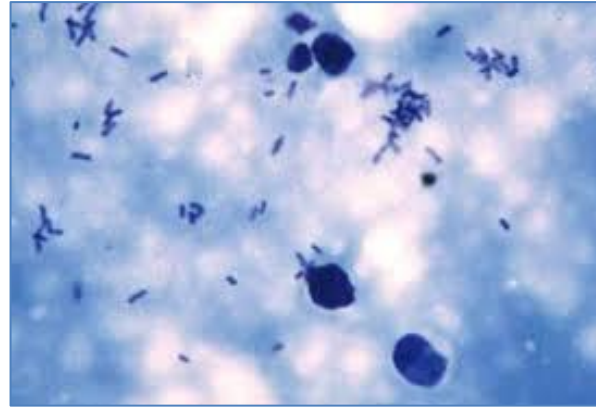
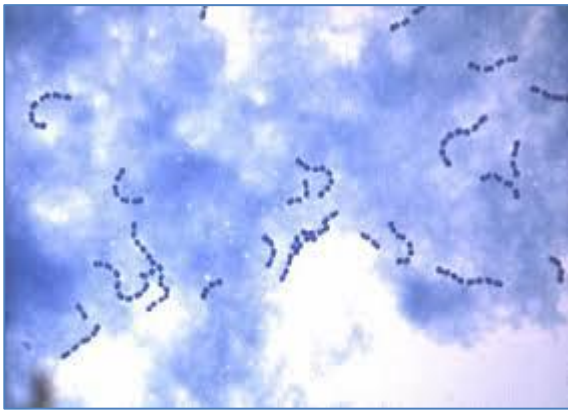
Microscopic Examination of Milk

- Microscopic examination of the milk is one of the most effective methods of rapid investigation of the presence of bacteria and leukocytes in milk.
- It is possible to determine the number and morphological characteristics of the causative bacteria in addition to the number of leukocytes (somatic cell) in milk, which are known as direct microscopic count (DMC) and direct microscopic somatic cell count (DMSCC) respectively.

Procedure

1. Milk sample was warmed to 40°C before making the smear using water path
2. Shake the sample 12-25 times, let to stand for about three mints until the foam disappears.
3. Transferred 0.01 ml of milk sample to clean glass slide using micropipette, spread over 1cm² areas marked on the slide using a marker pen.
4. The smear was spread evenly to perform a thin film that is fixed by drying on a 40°C clean level surface hot plate for five mints.
5. Stain the fixed-film using Levowitz-Weber Modification of the Newman-Lampert Stain solution for two mints, then let to stand on absorbent paper to dry.
6. After dryness the slide was rinsed in three-time changes in tap water at 37-45 °C, dried and stored in slide-storage box until examination.

Microscopic examination



Calculation

The number of fields counted for determination of somatic cell count

Average somatic cells	Number of fields
0-3	64
4-6	32
7-12	16
13-25	8
26-50	4
51-100	2
Over 100	1

*** Milk SCC is Calculated using the following formula:**



$$\textit{The DMSCC per ml} = \frac{\text{MF} \times \text{Average number per field}}{0.01}$$

Where MF(Microscopic factor) ≈ 3571
0.01 ml represents the volume of sample in milliliters