TESTS FOR MASTITIS DIAGNOSIS

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KEYWORDS: INTRODUCTION, IMPORTANCE OF MASTITIS DIAGNOSIS, SCREENING TESTS.

- Mastitis (Mammitis; Mammitte; Inflamation de la ubres)

A. Introduction:-

Mastitis is recognized the most costly Global problem of dairy/lactating herds caused by various Bacteria (Staphylococcus aureus, Streptococcus agalactiae, Streptococcus zooepidemics, Streptococcus faecalis, Streptococcus pyogenes, Streptococcus dysgalactiae, Corynbacterium bovis, Corynbacterium pyogenes, Klebsiella Spp., Salmonella Spp., Pseudotuberculosis, Mycobacterium bovis, Eschricia coli, Brucella abortus, Pasteurella multocida, Leptospira Pomona, Pseudomonas pyocyaneus etc.), Virus (Vesicular stomatitis, Infectious rhinotrachitis, Foot and Mouth disease, Pox virus infection etc.), Fungus (Trichosporon Spp., Aspergillus Spp., Candida Spp., Cryptococcus neoformans etc.), and Mycoplasma (Mycoplasma bovis, Mycoplasma bovigenetelium) that affect all breeds of dairy Cows, Buffaloes, Sheep, Goats and Horses but high yielding, exotic and cross breed Cows are highly susceptible to it. It is the disease of Udder and characterized by physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissues of the udder.

B. Importance of mastitis diagnosis:-

It is the disease of great economic importance for the dairy farmers as it is estimated that mastitis reduces milk by 21 % and Butter fat by 25 % in affected animal (Milk of the infected animal is of inferior quality and low in quantity), Decrease market value of cow, High cost of drugs to treat, and from the public health stand point this inferior quality milk is unfit for the human consumption and dangerous for health because many diseases (Tuberculosis, Brucellosis, Staphylococcal toxemia, Streptococcal sore throat, Scarlet fever and Gastroenteritis etc) can be transmitted via this milk. Therefore it is necessary to screen out the affected animal of the herd for timely treatment or culling as the disease is contagious in nature and can spread in the whole herd.
C. 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 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.

(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).

**Procedure:**

Milk is stripped directly into the chamber of plastic paddle with four chambers. Then the test reagent is added in the same ratio to each chamber. The milk and the reagent are rotated by movements of the paddle and the reaction is observed immediately. Positive milk sample will turn to greenish blue due to alkalinity. Due to presence of increased number of leukocytes a precipitate or gel is formed.

(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**

<table>
<thead>
<tr>
<th>Country</th>
<th>SCC / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>750,000</td>
</tr>
<tr>
<td>Australia</td>
<td>400,000</td>
</tr>
<tr>
<td>Canada</td>
<td>500,000</td>
</tr>
<tr>
<td>Pakistan</td>
<td>950,000 – 1200,000</td>
</tr>
</tbody>
</table>

**SCC**: Australia < Canada < United States < Pakistan

**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 500,000 cells per ml of milk is taken as +++ mastitis.
### Results interpretations for Somatic Cell Count / ml of milk

<table>
<thead>
<tr>
<th>SCC /ml of milk</th>
<th>% of quarter affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>300,000</td>
<td>6.2</td>
</tr>
<tr>
<td>400,000</td>
<td>12.8</td>
</tr>
<tr>
<td>750,000</td>
<td>24.3</td>
</tr>
<tr>
<td>1000,000</td>
<td>32.6</td>
</tr>
</tbody>
</table>

**(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: \( \text{AgNO}_3 + \text{Milk Chloride} = \text{AgCl-PPT} \)

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.

\( \text{AgNO}_3 + \text{K}_2\text{CrO}_4 = \text{Ag}_2\text{CrO}_4 \) (Brownish Red Silver Chromate)

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