

CURRENT TRENDS IN DIAGNOSIS OF MASTITIS: IN FIELD VETERINARY PRACTICES

R.P.DIWAKAR & VIBHA YADAV

Assistant Professor, Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry,
N.D. University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India

ABSTRACT

“Mastitis” is an inflammation of parenchyma of mammary gland which is characterized by physical, chemical and microbiological changes in milk and pathological changes in glandular tissue (Radostits *et al.*, 1994). Mastitis is occurring in most of the animals but major economic importance in cattles and buffaloes due to its effect on quality and quantity of milk in high yielder animals. To avoid the economic losses, it is important to identify the disease in early stage. Unlike the clinical form, in subclinical form there is neither visual detection of abnormalities in milk nor in mammary gland. So, routine diagnostic screening tests for early detection of mastitis are necessary to treat it and to avoid economic losses (Galdhar and Roy. 2003).

KEYWORDS: Mastitis, Inflammation

INTRODUCTION

Mastitis is occur in most of the animals but major economic importance in cattles and buffaloes due to its effect on quality and quantity of milk in high yielder animals “Mastitis” is an inflammation of parenchyma of mammary gland which is characterized by physical, chemical and microbiological changes in milk and pathological changes in glandular tissue.

For the diagnoses of mastitis following routine diagnostic tests are involves.....

- Physical examination of udder
- Strip cup test
- California mastitis test (CMT)
- Wisconsin mastitis test (WMT)
- White side test
- pH Determination test
- Chloride test
- Somatic cell count test (SCCT)
- Methylene blue reduction test (MBRT)

- Electrical conductivity Test(ECT)
- Milk Antitrypsin Assay
- Bromothymol blue test
- Bromocresol purple test
- Catalase test
- Hotis test
- Radial immunodiffusion test

Physical Examination of Udder

For detection of mastitis examination of mammary gland is important. Shape, size, consistency and contour of the udder should be viewed properly. Detail examination of teat and teat orifices should be made. Signs of inflammation viz. hot, swelling, pain, redness and loss of function should be clinically assessed. Each quarter should be classified according to the extent of abnormalities. The classification as suggested by Udaal (1964) may be followed. The udder is entirely normal. Varshney (2000) suggested that physical examination of the udder was informative about the size, shaped and consistency when conducted immediately after milking.

Strip Cup Test

Strip cup or strip plate test is routinely used in milking parlor for detection of clinical mastitis in individual animal and herd. In herd health management practices the milk machine operators visually examine the fore milk for gross abnormalities by squirting few stripes of milk on strip cup where abnormalities usually found are blood, flakes, clots and wateriness, which are suggestive of mastitis.

The use of strip cup test has some additional benefits apart from general identification of clinical mastitis as ____

- Stripping the first stream of milk stimulates milk letdown, resulting in faster milk out.
- Fore milk is higher in bacteria than subsequent milk, removal of this milk may reduce bacterial contamination of the milking machine and udder.

California Mastitis Test (CMT)

California mastitis test is simple, inexpensive and rapid screening test. It estimates the number of somatic cells present in milk. Somatic cells are composed of approximately 75% of leucocytes (WBC) and 25% of epithelial cells (secretory and lining cells). As the leucocytes are the bodies of primary defense mechanism, its increase in mastitis is enviable. However, rise in epithelial cells are due to injury to mammary epithelium (Galdhar *et al.*, 2004).

CMT test is conducted by mixing the test reagent (CMT reagent) with an equal quantity of milk. It reacts with DNA of the nuclei of the somatic cells in the milk to form a gel. The reaction is then visually scored as 0, T (Trace), 1, 2 or 3 depending upon the amount of gel that forms. Formation of more gel indicates higher somatic cell count. There is correlation in between CMT score and average somatic cell counts as present in table 1.

Table 1: Correlation in Between CMT Scores and SCC

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

Wisconsin Mastitis Test (WMT)

Wisconsin mastitis test is primarily a laboratory test and generally conducted on bulk tank milk samples. In both tests (WMT and CMT) same type of reagent is used. In CMT the test result reaction is qualitatively estimated while in WMT the test result reaction are measured (mm).

The test is conducted by combining a measured quantity of milk with equal amount of reagent. The milk and reagent are then mixed for 8 to 10 seconds. The mixture is drained for a period of 18 seconds and returned to an upright position. After waiting one minute, the amount of fluid remaining in the tube is measured. WMT scores are generally calculated in millimeters (mm) and used to predict the average number of somatic cell present in the milk. There is relation in between WMT scores and SCC (Table 2)

Table 2: Relationship between WMT Scores and SCC

WMT (mm)	SCC	WMT (mm)	SCC
3	140	21	990
4	165	22	1050
5	195	23	1130
6	225	24	1700
7	260	25	1280
8	300	26	1360
9	340	27	1440
10	380	28	1525
11	420	29	1610
12	465	30	1700
13	515	31	1800
14	565	32	1930
15	620	33	2030
16	675	34	2180
17	730	35	2280
18	790	-	-
19	855	-	-
20	920	-	-

White Side Test

This test depends upon the increased leukocyte count in the milk. In mastitis, test result denotes white flakes formation while in normal milk test result shows formation of milky opaque fluid.

PH Determination Test

The normal milk pH is 6.5-6.8 and isotonic with blood plasma. In mastitis, during late lactation and dry period, the concentration of lactose and casein in the milk is reduced whereas sodium chloride and sodium bicarbonate pass in to the alveoli from plasma to maintain isotonicity. So, during such situations milk becomes alkaline with greater amount of

chlorides.

Chloride Test

This test denotes presence of increased quantity of chloride in mastitis milk. Normal milk contains about 0.07% of chlorides. Davis (1999) reported that in mastitis there is decreased amount of lactose and increased amount of sodium chloride to maintain the milk osmotic pressure hence during inflammation there is increase in the chloride content (more 0.14%).

Somatic Cell Count Test (SCCT)

The count used includes the direct microscopic somatic cell count (DMSCC), the bulk milk somatic cell count (BMSCC) and individual cow somatic cell count (ICSCC).

In direct microscopic somatic cell counts, milk smear was made on a clean glass slide in the area of 1cm² stained with 1% methylene blue and 60 fields were examined for the count. The average number of cells per field was multiplied by the multiplication factor of the microscope. The value so obtained is considered equal to the number of cells/ml of milk sample.

Now day's uses of electronic somatic cell counters are practiced for DMSCC, which has following advantages...

- The procedure can be automated thus allowing centralization of laboratory procedures.
- Preserved samples can be used for counting.
- The procedure is more precise and more objective.
- The primary disadvantage of electronic somatic cell count procedures is requirement of costly equipment with trained workers.

Radostits *et al* (1994) reported SCC more than 250000/ml were considered to be indicative of inflammation, whereas count less than 100,000/ml was indicative of normal udder and counts more than 500,000cells/ml was indicative of infection.

Bulk milk somatic cell count is the universally adapted screening test for the mastitis. Benefit of this test is that it creates awareness in the mind of farmer/dairy owners observers about existence of mastitis problem in herd.

Methylene Blue Reduction Test (MBRT)

Methylene blue reduction test (MBRT) measures chemical activities, especially the respiratory activities of the bacteria in the milk. The cause of the reaction is the micro organisms in the milk first removes the oxygen by respiration and when all oxygen and some other reducible components of the milk has been removed or reduced, this changes color of methylene blue to methylene white (Davis,1999).

Electrical Conductivity Test (ECT)

The conductivity of milk is defined by the substances in solution which can ionize and which there conduct an electrical current. When the concentration of sodium chloride rise, the conductivity rises proportionately. Therefore, measurement of electrical conductivity is used as a simple physical method to diagnose mastitis. Electrical conductivity of

milk can be determined by using a hand held (portable) electrical conductivity meter (milk checker or digital mastitis detector). The EC of milk expressed in the unit of milk seimens/cm.

This test has following advantages:

- No specific training is needed.
- Easy to do and result are readily available.
- Useful in finding out the etiology also.

Milk Antitrypsin Assay

Milk antitrypsin activity is due to the leakage of blood alfa-1 Protease inhibitor in to milk represents increased permeability. Samad and Awaz (1996) reported normal milk contains < 200 BEN unit/ml of trypsin inhibitor. However in mastitis, this was increased to many folds and the trypsin inhibitor test appear to be superior over CMT in diagnosing sub-clinical mastitis.

This test has following advantages:

- Easy perform and cheap.
- No specific training is needed.
- Result find out within 5 minutes.

The only drawback is that the etiologies cannot be identified. But Easy practice under field condition. They are certain factor which affects antitrypsin activity in the milk like

- High level of antitrypsin is seen in colostrums and decrease within a few days after calving.
- **Lactation stage:** - The effect is evident in early and in the end of lactation and its level increased when mammary gland is infect.

Bromothymol Blue Test (BTB)

The reaction of the milk can be tested by this method. pH of the mastitis milk is alkaline in reaction. The Ph changes from normal 6.4 to 6.8 to a pH of nearly 7.4.

This test has been successfully used to diagnose mastitis (Chanda *et al.*, 1989). For this B.T.B. card test papers 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 g) bromothymol blue in 100 ml ethanol at 4 different spots on the paper and indicate as left for (LF), Left hind (LH), Right for (RF) and Right hind (RH). One drop of suspected milk has to be put directly on the indicator spot and changes of the colour are to be noted. The changes of the colour may be scored as: (Pale green) i.e., normal quarter and “+”, “++”, “+++” (according to changes in colour from moderate green to dark blue green). The only disadvantage of this test is that cows in latter stages of lactation may give false positive reaction.

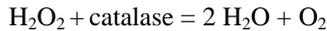
Bromocresol Purple Test

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 to be added to 3 ml of milk. Normal milk following addition of solution will appear as yellow while mastitis milk appears as blue or purple.

Catalase Test

There is presence of catalase in every living cell. Leucocytes contain increase quantity of enzyme. The determination of catalase will give fair indication of the presence of leucocytes. In udder infection, the leucocyte number is increased. Therefore, catalase determination indicates about the presence or absence of infection. The amount of catalase present is determined by the ability to break down hydrogen peroxide to oxygen and water. For this test 1% H₂O₂ is used.



Hotis Test

This is help to detect the presence of *Streptococcus agalactiae*. *Streptococcus agalactiae* ferment the lactose of milk rendering it acidic. Thus, the indicator bromocresol purple turn to yellow.

0.5ml of 0.5% aqueous bromocresol purple solution is mixed to 9.5 ml of milk in a sterile test tube. This is mixed thoroughly and incubated at 37°C for 24hs. *Streptococcus agalactiae*, if presence in the milk will produce canary yellow colonies along the side of the test tube.

Radial Immunodiffusion Test

Radial immunodiffusion test helps to assess the integrity of mammary mucosa. This test determines the concentration of serum albumin of milk.

CONCLUSIONS

For diagnosis of Mastitis in Field Veterinary Practices most important is to diagnose early stage of disease so that to reduce the economical losses to dairy farmers and poor farmers via above said test. Mastitis is caused by almost all the bacteria directly or indirectly.

REFERENCES

1. Chanda *et al.*, (1989). Indian vety.K,66:571
2. Davis JG. (1999). Milk testing (The laboratory control of milk) Agrobotanical publishers, Bikaner, India.
3. Galdhar CN and Roy S. (2003). Recent trends in therapeutic management of mastitis. *Journal of Remount Veterinary Corps*.42 (1):5-11.
4. Galdhar CN, Gaikwad RV, Samad A, Keskar DV, Bhalerao DP, Dighe DG, Dighe DG and Zende RJ. (2004). Bovine udder and its immunity- An Update. *Journal of Remount Veterinary Corps*.42 (4):179-185.
5. Radostits OM, Blood DC and Gay CC. (1994). Veterinary Medicine, 8th Edn., Bailliere Tineal, London.
6. Samad A and Awaz KB.1996. Trypsin inhibitor- quantitation. *Journal of Bambay Veterinary College*.4:25-31
7. Varshney JP (2000). Proceeding of Indian Veterinary Congress, Round table conference on mastitis. I.V.R.I., Izatnagar. February 18-19, 2000.Pp 1-5.