

Comparative evaluation of three tests used for the screening of mastitis

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Bovine mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk production, treatment costs, increased labor, milk withheld following treatment, death, and premature culling (Kaneene and Hurd 1990, Miller *et al.* 1993). Diagnosis of mastitis in the early stage is important to reduce losses and enhance recovery. Early diagnosis of mastitis is vital for control. International Dairy Federation (IDF) have been approved the somatic cell counts (SCC) and microbiological status of the quarter for diagnosis of mastitis. Bacteriological culture of milk samples is the standard method for identifying mastitis. Milk culture identify presence of pathogens but does not measure the degree of inflammation associated with the infection.

California mastitis test (CMT) and sodium lauryl sulphate test (SLST) are reliable cow-side screening tests for sub clinical mastitis. These tests are based upon the amount of cellular nuclear protein present in the milk sample. Modern mastitis tests allow for indirect determination of the number of somatic cells in milk (Greiner *et al.* 2000). As the inflammatory process develops in udder tissue the number of these cells (particularly leucocytes) in milk sharply increase. SCC, CMT and intramammary infection (IMI) are associated significantly, therefore these parameters provide necessary information to evaluate udder health status in cows. Present study aimed to compare indirect mastitis diagnostic tests for their ability to classify correctly udder health status of individual cows.

The present study was carried out on organized dairy farm. A total of 85 cows of different age groups, parities and stage of lactation from dairy herds were studied. Cows were housed in tie stall barns and milked twice in a day by hand. All teats were washed with potassium permanganate solution before milking by milkers. Milk samples (325) were collected after proper disinfection of teats with 70% ethyl alcohol, 60 ml of milk samples from all four quarters, viz. left fore (LF), left hind (LH), right fore (RF) and right hind (RH) were collected

aseptically after squirting few streams, in separate sterile vials. CMT was conducted at the spot before collection of milk sample. The milk samples were kept in an ice box and carried to the laboratory. Samples were stored at 4 to 10°C till further investigation.

Milk samples from apparently healthy cows were screened by CMT, SLST and SCC. CMT was performed cow-side and according to manufacturer's recommendations on quarter foremilk samples. In brief, 3 ml of milk sample was added with equal quantity of CMT reagent in each cup of CMT paddle and rotate for few seconds and record the result within 30 seconds. CMT results were recorded as 0 (negative), T (trace), 1 (+), 2 (++), or 3 (+++). SLST as per Pandit and Mehta (1969) and procedure is same as CMT. SCC was performed as per Schalm *et al.* (1971). Calculations of diagnostic test characteristics were performed using the milk bacteriological culture (Quinn *et al.* 1994) result as a gold standard control.

Percent accuracy of the tests and sensitivity, specificity, and predictive values of CMT, SLST and SCC results, compared to culture, were calculated using standard two-by-two contingency tables. Correlations between the dependent variables were calculated using Pearson's correlation.

A total of 325 milk samples from crossbred dairy cows were subjected to CMT, SLST, and SCC. When CMT and SLST scores were >1+ and SCC value >2×10⁵/ml of milk (threshold value). The 220 and 105 milk samples were CMT positive and negative respectively. Whereas, 253 and 175 milk samples were positive by SLST and SCC, respectively. Per cent accuracy of CMT, SLST and SCC were 75.69, 51.38 and 92.00% (Table 1). In this study SCC was most reliable and closest to bacteriological culture indicating inflammatory condition of the udder. Mastitis caused by pathogenic bacteria leads to increased level of leukocytes in milk, which improved the accuracy of SCC. However, SCC increased in the first few days of lactation and may remain high up to the first month of lactation (Atakan 2008) and again increased towards the end of lactation as normal physiological condition. Sederevicius *et al.* (2006) reported a temporary

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Table 1. Percentage accuracy of various indirect tests used for the diagnosis of mastitis

Tests	Samples examined	Positive samples	TP	FP	TN	FN	Accuracy (%)
CMT	325	220 (67.69)	168 (76.36)	52 (23.63)	78 (74.28)	27 (25.71)	75.69
SLST	325	253 (77.85)	145 (57.31)	108 (42.69)	22 (30.55)	50 (69.44)	51.38
SCC	325	175 (53.85)	172 (98.28)	3 (1.71)	127 (84.67)	23 (15.33)	92.00

Values in parenthesis are in percentage; TP, true positive; FP, false positive; TN, true negative; FN, false negative.

Table 2. Agreement and correlation of various indirect tests used for the diagnosis of mastitis with bacteriological (cultural) examination

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR (%)	NLR	□	r	p
CMT	86.20	60.00	76.4	74.3	2.15	0.23	0.47	48.35	-0.00
SLST	74.40	16.90	57.3	30.6	0.89	1.51	-0.09	10.28	0.06
SCC	88.20	97.70	98.3	84.7	38.22	0.12	0.84	84.41	0.00

PPV, Positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; k, kappa (measure of agreement); r, Pearson's correlation.

increase in SCC just after calving due to adaptation of the udder from non-lactating to lactating status. While in mid lactations SCC usually remains in normal range. High SCC in milk reduces quality (shelf life and flavour) of both milk and dairy products (cheese) and butterfat yield. SCC increases due to inflammation or udder infection. The positive reaction of CMT and SLST depend on the concentration of somatic cells in the milk. SCC like culture is not easy to perform and needs a skilled hand, adequate laboratory facilities and time. Automatic digital somatic cell counters are available in the market.

In accuracy CMT was at third position but easy, field based and sufficient to diagnose pre-clinical mastitis. Detergent in CMT and SLST dissolves or disrupts outer cell wall and the nuclear cell wall of leukocyte (somatic cells). DNA released from somatic cells and gel to form a stringy mass. As the number of leucocytes increase in a quarter, the amount of gel formation increase in a linear fashion. Grading of CMT and SLST results are based on gel formation. Out of 220 CMT positive milk samples, 195 samples were positive by cultural isolation.

Sensitivity and specificity of SCC was higher than CMT and SLST (Table 2). The specificity of SLST was very low (17.16%). SCC showed more correlation (Pearson's) with bacterial culture followed by CMT and SLST. SCC had excellent agreement with bacterial culture and CMT had fair to good agreement (Table 2). It is considered that kappa values lower than 0.4 represent poor agreement, values between 0.4 and 0.75 fair to good agreement, and values higher than 0.75 excellent agreements. The positive and negative predictive values and likelihood ratios are given in

Table 2. Present findings are in agreement with Sharma *et al.* (2008), they reported that SCC was most accurate test for the diagnosis of sub clinical mastitis followed by modified CMT and Modified White Side test (MWST). Patel *et al.* (2000) reported higher reliability of CMT (85.6%) followed by MWST (79.7%). Reddy *et al.* (1998) compared the specificity and sensitivity of CMT and SCC with cultural and observed 100% predictive value with the cultural test of the milk, 84.84% specificity for SCC and 73.30% for CMT. Tanwar *et al.* (2001) also compared various diagnostic tests for detection of SCM and indicated 100 and 96% sensitivity for SCC and CMT respectively. According to Goswami *et al.* (2003), animal-wise efficacy of indirect tests taking cultural examination as a standard was in the following descending order SCC (97.46%), MCMT (69.62%) and MWST (63.29%). Present findings on accuracy and sensitivity of indirect tests for detection of SCM supported the earlier findings. Barbosa *et al.* (2002) reported that the SCC and CMT are dependent and have high correlation for the diagnosis of SCM. Sudhan *et al.* (2005) reported that the percent of agreement of CMT, SLST, BTB card test and WST with bacteriological examination were 57.89, 62.07, 64.46 and 68.65% respectively. The present study suggests that CMT could be used as regular mastitis screening test in the field condition, even by non-technical or less trained dairymen's.

SUMMARY

The present study was conducted on 325 milk samples to screen the dairy cows for mastitis by CMT, SLST and SCC. The 220 and 105 milk samples were CMT positive and

negative respectively. Whereas, 253 and 175 milk samples were positive by SLST and SCC, respectively. Per cent accuracy of CMT, SLST and SCC were 75.69, 51.38 and 92.00%. In this study SCC was most reliable and closest to bacteriological culture indicating inflammatory condition of the udder. In summary, CMT was most accurate after SCC. CMT is the reliable diagnostic method in field conditions.

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