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# Comparison of California mastitis test and Draminski mastitis detector as on-farm methods for monitoring udder health in lactating buffalo

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**Abstract**

   This study was conducted to compare the performance of the California Mastitis Test (CMT) and Draminski Mastitis Detector (DMD) for monitoring udder health in lactating buffalo. A total of 647 milk samples collected from 145 apparently healthy lactating buffalo cows during the first three months of the lactation period were used in this study. Both DMD and CMT were performed on-farm directly after sampling of the milk. The diagnostic accuracy of CMT and DMD was evaluated by estimating the area under the Receiver-Operating Characteristic curve (AUC) based on a value of 200,000 somatic cells per mL as a cut-point value for an infected quarter detected by direct microscopic somatic cell count. Data analysis showed that 63.76% of CMT results were "Trace" and 99% of DMD readings were greater than 300. The accuracy of CMT and DMD was close to each other; the AUC was 80.1% and 81.4% for CMT and DMD, respectively. The optimal cut-point value for CMT was Trace (T), and 515 for DMD. In conclusion, both CMT and DMD are practical on-farm tests for identifying infected udder in buffalo cows, and the suggested cut-point value for DMD is 515 for buffalo's milk testing instead of the 300 that was assigned by the manufacturer for cow's milk.

## Introduction

   Monitoring mammary gland health in lactating dairy animals is one of important measures for dairy farmers to ensure milk production profitability (1). Diagnosis of clinical mastitis is considered easy as it is detectable by the physical examination, in contrast to the subclinical mastitis that is accomplished by the use of direct or indirect tests on-farm or at laboratory (2). Although economic losses due to clinical episodes are basically due to cost of treatment and discarded milk, subclinical mastitis causes long-term decrease of milk production (3). On-farm diagnostic tests are used worldwide for several decades in monitoring mammary gland health in lactating dairy animals. It might be difficult for regular monitoring of udder health to perform bacteriological culture or molecular diagnosis as they are time-consuming and require professional laboratory equipment, although they are preferred methods for identification of the causative pathogens of mastitis (4). Therefore, on-farm tests are applied as quick and affordable screening tests with accuracy that can assist veterinarians and practitioners in the diagnosis and determination of the treatment plan (5). Such tests are based on indirect somatic cell count, whereas others tests are based on the electrical conductivity of the milk (6). California mastitis test (CMT) is considered the most common test used as an indirect method to semi-quantitatively predict the somatic cell count (SCC) in milk (5). It has been found that all quarters with SCC greater than 200,000 cell/mL had intramammary infection, while about 98% of those quarters with SCC less than 200,000 cell/mL were not infected (7).

It was developed and first described by Schalm and Noorlander in 1957 (8) with a principle based on destruction of the somatic cells' membranes due to the action of the test reagent allowing gel formation as a result of the reaction between the test reagent and DNA of the cells (6). Somatic cells normally exist in normal milk consisting mainly from epithelial cells and some leukocytes as a function of mammary epithelium desquamation; however, the number of leukocytes is increased indicating an inflammatory process inside the mammary gland (9). Draminski Mastitis Detector (DMD) is probably the most recent hand-held device used for monitoring mammary gland health status developed by Mr. Janusz Dramiński, the president of Draminski Company, in 1989 with a principle based on the measure of milk electrical resistance (10). In brief, the infection damages the mammary gland cells leading to an increase of $Na^+$ and $Cl^+$ and a decrease of $K^+$ and lactose concentrations which can cause a decrease in the resistance of milk to the electrical current (11).

Although the measure of electrical conductivity (the reciprocal of the resistance) of milk as an indicator of intra-mammary infection is not new and does not have high accuracy compared to those for SCC, it could be promising with the development of new technology and hand-held devices (6). Performance of CMT and DMD has been compared in cows. For instance, Galfi *et al.* (12) compared results of CMT and DMD with bacteriological culture and found that the accuracy of CMT was higher than DMD, 80% and 52%, respectively. On the other hand, Iraguha *et al.* (13) compared the use of four tests including CMT and DMD, and found a moderate association between the four tests; however, they did not report the agreement between both CMT and DMD. In buffalo, limited knowledge is available except that for Kala *et al.* (14) results who revealed that the percentage of accuracy for CMT and DMD was 89.83% and 77.27%, respectively, compared to bacteriological culture. It is important to perform more studies on buffalo; particularly DMD reports higher readings in fatty milk (10). Therefore, the objective of the study conducted here was to compare the performance of CMT and DMD for monitoring udder health in lactating buffalo.

## Materials and methods

### Ethical approval

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the College of Veterinary Medicine, University of Mosul, with ethical approval number UM.VET.2021.056.

### Study animals and samples

This study was conducted using 145 recently calved lactating buffalo cows owned by private owners distributed around the Tigris River in Nineveh Governorate, north of Iraq. A total of 647 milk samples were used in this study collected between Oct $1^{st}$, 2021 and June $30^{th}$, 2022 during the first three months of lactation. Prior to each sampling, the udder was cleaned and the teat orifice was sterilized.

### Draminski mastitis detector (DMD)

For this purpose, DRAMINSKI® Mastitis Detector 1Q was used according to the manufacturer's instructions (Dramiński S. A., Gietrzwałd Poland). In brief, approximately 20 mL of first foremilk was directly collected from the teat to the measuring cup of DMD 1Q, the detector was turned on and the electrical resistance value displayed on the screen of the detector was recorded (10).

### California mastitis test (CMT)

For this purpose, the CMT kit (ImmuCell Co., Portland, ME, USA) was used according to the manufacturer's instructions. An additional amount to the previous 20 mL of milk was collected. Approximately 3 mL of milk was poured into the CMT puddle and an equal amount of the CMT reagent was mixed by the gentle continuous rotate movement of the puddle in a horizontal position for 30 seconds. The results were recorded as the following: (0; negative): when the mixture remained liquid with no evidence of precipitate or gel formation, (T; Trace) when a slight precipitate was formed and disappeared with continuous movement, (1+; weak positive) when a precipitate was formed without gel formation tendency, (2+; distinct positive) when the mixture was immediately thickened with gel formation tendency, and (3+; strong positive) when a distinct gel was formed (8).

### Laboratory examination

The remaining milk sampled for field tests was kept cooled in plastic vials and transferred to the laboratory for direct microscopic somatic cell count (DMSCC).

### Preparation and staining of milk films

The milk vials were gently shaken to mix the milk, 0.01 mL of milk was then withdrawn by micropipette and spread evenly over an area of 1 $cm^2$ delineated on a slide, left to completely dry, and then stained with the Newman-Lampert stain. The stain was manually prepared according to the components of 01375 Newman's Stain Solution, modified (Merck KGaA, Darmstadt, Germany) as the following: 0.6 g methylene blue chloride, 52 mL ethyl alcohol 95%, 44 mL Tetrachlorethane, and 4 mL Glacial acetic acid) (15).

### Counting of somatic cells

Each slide was examined under an oil-immersion objective. Every identifiable stained polymorphonucleated or intact mononucleated cell was counted in a total of 30 fields around the entire film. The average of cells counted in 30 fields was multiplied by the microscopic factor calculated according to the following equation (8) [microscopic factor=

area of the film x (area of the field x volume of the film)]. Where the area of the film = 100 mm, the area of the field for the used microscope = $\pi r^2$ (3.1416 × 0.0081), and the volume of the film = 0.01 mm.

**Statistical analysis**

The ability of conducted tests to identify infected quarter was measured according to the following: (i) proportion of those identified by DMSCC with > 200,000 cells/mL (9), (ii) proportions of those identified by CMT (4), and (iii) the proportion of those identified by DMD with electrical resistance less than 300 (DMD manual). The direction and strength of the linear correlation between CMT, DMD, and DMSCC were measured by Pearson's correlation; Pearson's *r* (16,17). The diagnostic accuracy of CMT and DMD was evaluated by estimating the area under the Receiver-Operating Characteristic (ROC) curve; AUC (18). In this analysis, the value of 200,000 somatic cells per mL is the cut-point value for an infected quarter (9), and the smaller DMD readings indicated more positive results because the resistance of milk to the electrical current decreases with the infection (11). In addition, the optimal cut-point values for CMT and DMD were considered as the minimum value for the absolute difference between the sensitivity and specificity obtained from the ROC curve (19). The diagnostic sensitivity (Se) and specificity (Sp) of optimal cut-point for CMT and DMD were reported. Finally, data analysis was conducted by use of STATA 13.0 - StataCorp, College Station, TX (20), and IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA) with the aid of Excel spreadsheet 2010 (Microsoft Corp., Redmond, WA, USA).

**Results**

Results of DMSCC, CMT, and DMD were summarized (Table 1). Data analysis revealed a positive correlation between DMSCC and CMT readings (*r* = 0.629, P<0.01), and negative correlation between DMSCC and both DMD and CMT and DRM; (*r* = − 0.405 and − 0.555, P<0.01), respectively (Figures 1-5). The CMT showed more accuracy than DMD, AUC = 0.801 and 0.814, respectively (Figures 4 and 5), considering SCC > 200,000 cells/ml is the cut-point for positive/negative quarter. ROC analysis indicated that the optimal cut-point for CMT was (T: Trace) with values of Se

= 81.5% and Sp = 77.5% (Table 2). On the other hand, the optimal cut-point for DMD was 515 with values of Se = 73.2% and Sp = 71.5% (Table 1). The analysis revealed that the assigned cut-point for DMD; i.e., 300, had Se value of 5.2% and Sp 100%.

Table 1: Results of DMSCC, CMT, and DMD in a total of 647 milk samples from apparently healthy lactating buffalo cows

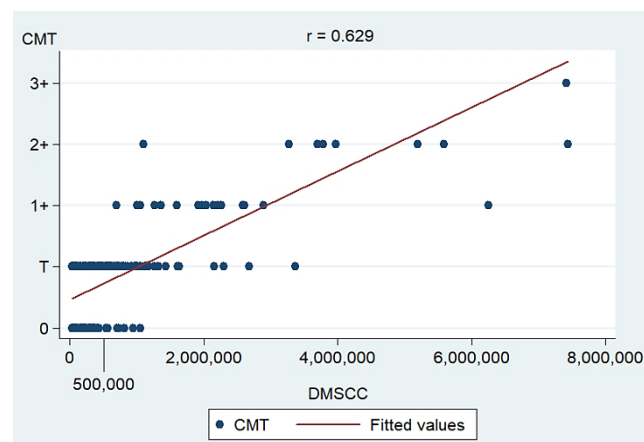| Reading of the test | N | % |
| --- | --- | --- |
| DMSCC | | |
| > 200,000 cells / mL milk | 496 | 76.66 |
| ≤ 200,000 cells / mL milk | 151 | 23.34 |
| CMT | | |
| 0: Negative | 208 | 32.35 |
| T: Trace | 410 | 63.76 |
| 1+: Weak positive | 16 | 2.49 |
| 2+: Distinct positive | 8 | 1.24 |
| 3+: Strong positive | 1 | 0.16 |
| DMD | | |
| ≥ 300 | 641 | 99.07 |
| < 300 | 6 | 0.93 |



Figure 1: Correlation between CMT results and somatic cell counts counted by DMSCC in milk from lactating buffaloes, where: CMT = California Mastitis Test, DMSCC = Direct Microscopic Somatic Cell Count, the direction = positive, the strength = 0.629.

Table 2: The ROC analysis of CMT and DMD for detection of intra-mammary infection based of DMSCC > 200,000 cells/mL

| Test | Area Under ROC curve (AUC) | | | Cut-point value | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Value | SE | 95% CI | Value | Se | Sp |
| CMT | 0.801 | 0.021 | 0.759, 0.842 | T | 81.5% | 77.5% |
| DMD | 0.814 | 0.019 | 0.776, 0.852 | 515 | 73.2% | 71.5% |

Abbreviations: SE = Standard Error, CI = Confidence Interval, Se = Sensitivity, Sp = Specificity.
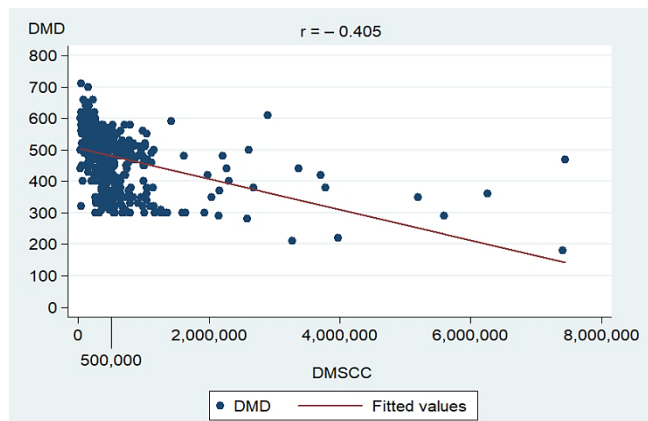
Figure 2: Correlation between DMD results and somatic cell counts counted by DMSCC in milk from lactating buffaloes, where: DMD = Draminski Mastitis Detector, DMSCC = Direct Microscopic Somatic Cell Count, the direction = negative, the strength = – 0.405.
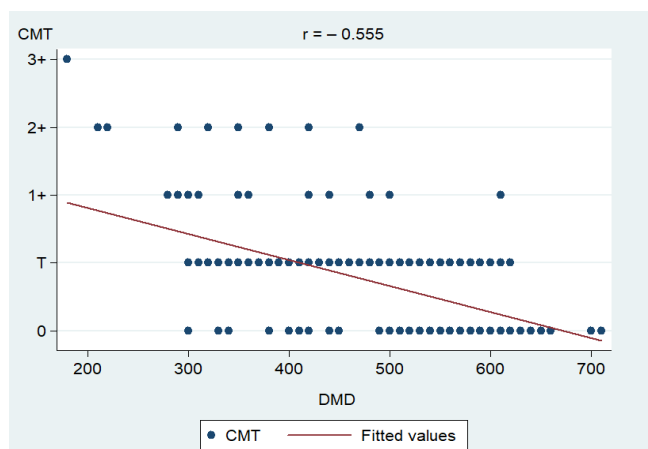


Figure 3: Correlation between DMD results and CMT testing milk from lactating buffaloes, where: DMD = Draminski Mastitis Detector, CMT = California Mastitis Test, the direction = negative, the strength = – 0.555.

**Discussion**

It is crucial to early detect intramammary infection in dairy animals as accurately as possible (21). Several on-farm methods can be used to detect the intramammary infection with different identified accuracy (22,23). The study conducted here has examined the performance of CMT and DMD as on-farm methods for identification of intra-mammary infection in lactating buffalo cows. The results of both CMT and DMD have been compared to the DMSCC as a laboratory indicator for intra-mammary infection. This is the first local study used DMD in buffalo milk and evaluated its cut-point value.
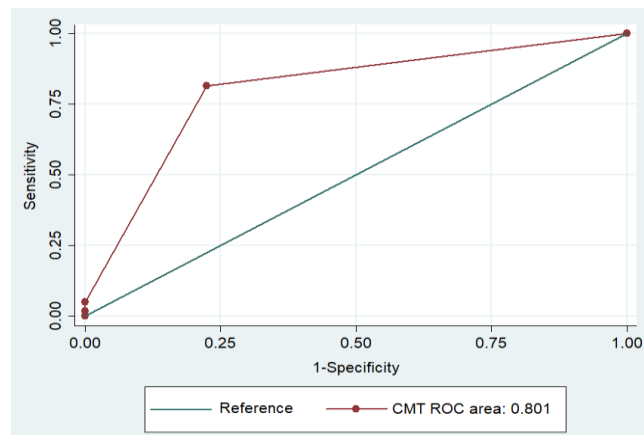


Figure 4: Area under the ROC curve (AUC) for CMT as a method for identification of intra-mammary infection in lactating buffaloes, assuming DMSCC as a gold standard with a cut-point 200,000 cells/mL.
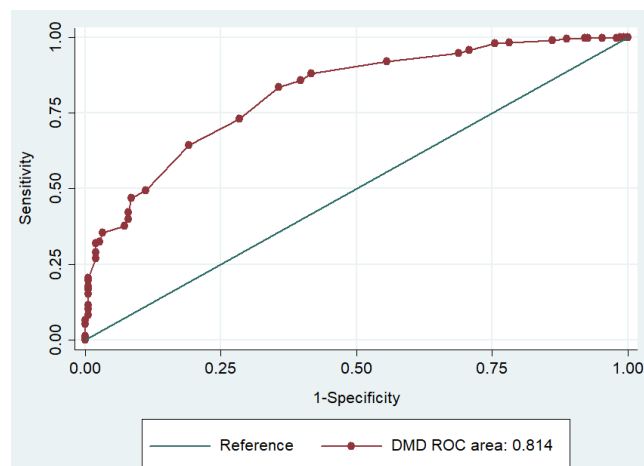


Figure 5: Area under the ROC curve (AUC) for DMD as a method for identification of intra-mammary infection in lactating buffaloes, assuming DMSCC as a gold standard with a cut-point 200,000 cells/mL.

In this study, a positive correlation was observed between CMT and DMSCC. This is an understandable result because the principle of CMT is based on the destruction of the somatic cells' membrane due to the action of the test reagent; therefore, as the SCC increases the gel formation as a result of the reaction between the test reagent and DNA of the cells becomes more distinct or strong indicating an increase in leukocytes as a function of the inflammatory process inside the mammary gland (6, 9). However, the strength of the correlation between CMT and DMSCC was only 0.629 which could be attributable to false positive and false negative results of CMT. False positive and false negative

results were observed in milk samples from buffaloes (14) as well as cows (12).

The study conducted here observed a negative correlation between DMD and both CMT and DMSCC. This result is attributable to the difference in the principle of DMD from that of CMT and DMSCC. That is, the DMD principle is based on the measure of milk electrical resistance which decreases with the infection as a function of an increase of $Na^+$ and $Cl^+$ and a decrease of $K^+$ and lactose concentration (11), whereas CMT is based on an increase of SCC in the infected mammary gland (6). This result suggests that considering the correlation coefficient only in the comparison of DMD and CMT results is not enough.

In this study, the ROC curve was used to compare the performance of CMT and DMD as well as to examine overall their diagnostic performance (24). The analysis indicated that CMT and DMD had close accuracy. In a study on dairy cows, the accuracy of CMT was greater than the hand-held milk electrical conductivity meter when study cows were classified based on SCC > 200,000 cells/mL (25). In that study, however, the hand-held milk electrical conductivity meter was different from that of our study. In addition, the accuracy of both tests was lower than that detected in our study, which could be attributable to the difference in the species of animals used in both studies; buffalo versus cows. Finally, the sensitivity and specificity of revealed in our study for both CMT and DMD were in line with previous studies on buffalo (10) and cows (12).

The ROC analysis conducted here revealed that the optimal cut-point of CMT was CMT was (T; Trace) with values of Se = 81.5% and Sp = 77.5% indicating that the category "Trace" for CMT is better to be used a cut-point value, which was suggested having 150,000 to 500,000 cells/mL (8). On the other hand, the optimal cut-point for DMD was 515 with values of Se = 73.2% and Sp = 71.5%. This value is greater than assigned by DMD the manufacturer; i.e., 300. One reason is that buffalo milk has more fat than cows. According to the DMD manual, fatty milk increases the magnitude of DMD readings above the average. Nevertheless, further studies are required to confirm this result, particularly using bacteriological culture rather than SCC.

## Conclusions

The study concluded that CMT and DMD are practical on-farm tests for identifying infected udder in lactating buffalo cows. Draminski mastitis detector has substantial accuracy in detecting infected udder in buffalo; however, the cut-point value of 300 assigned by the manufacturer should be used with caution in buffalo milk, with a suggestion of using 515 as an alternative value for buffalo milk.

## Conflict of interest

The authors declare that there is no conflict of interest in the research.

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# مقارنة اختبار كاليفورنيا وكاشف درامينسكي لالتهاب الضرع كطرائق حقلية لمراقبة صحة الضرع في الجاموس الحلوب

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## الخلاصة

أجريت الدراسة الحالية لمقارنة أداء اختبار كاليفورنيا وكاشف درامنيسكي لالتهاب الضرع لمراقبة صحة الضرع في الجاموس الحلوب. تم استخدام ٦٤٧ عينة من الحليب جُمعت من ١٤٥ إنثى من إناث الجاموس سليمة سريرياً خلال الأشهر الثلاثة الأولى من فترة إنتاج الحليب. تم تطبيق اختبار كاليفورنيا وكاشف درامنيسكي في الحقل بعد جمع عينات الحليب مباشرة، وتم تقييم الدقة التشخيصية للاختبارين من خلال تقدير المنطقة الواقعة تحت منحنى خاصية عَمَل المستقبِل باعتماد قيمة ٢٠٠,٠٠٠ خلية جسدية لكل مليلتر بوصفها حداً فاصلاً بين الضرع المصاب وغير المصاب تم حسابها عن طريق العد المجهري المباشر للخلايا الجسدية. أظهر تحليل البيانات أن ٦٣,٧٦% من نتائج اختبار كاليفورنيا كانت "أثر زهيد"، وأن ٩٩% من قراءات كاشف درامنيسكي كانت أعلى من ٣٠٠. وبيّنت النتائج أن دقة اختبار كاليفورنيا وكاشف درامنيسكي كانت قريبة من بعضهما البعض، إذ بلغت قيمة المنطقة الواقعة تحت منحنى خاصية عَمَل المستقبِل ٨٠,١ و ٨١,٤%، على التوالي، وأن القيمة المثلى للحد الفاصل لاختبار كاليفورنيا هو أثر زهيد و ٥١٥ لكاشف درامنيسكي. نستنتج من الدراسة الحالية أن اختبار كاليفورنيا وكاشف درامنيسكي اختبارين حقليين عمليين لتحديد الضرع المصاب في إناث الجاموس، مع اقتراح القراءة ٥١٥ حداً فاصلاً لكاشف درامنيسكي لاختبار حليب الجاموس بدلاً من ٣٠٠ التي تم تعيينها من قبل الشركة المصنعة لحليب الأبقار.