

Correlation between some direct and indirect tests for screen detection of subclinical mastitis

Kamal, R. M., Bayoumi, M. A. and *Abd El Aal, S. F. A.

Food control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

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Abstract

This study was undertaken to investigate the effectiveness of California mastitis test (CMT), white side test (WST), chlorine test and electrical conductivity (EC) in the diagnosis of subclinical mastitis in dairy cows in a comparison to somatic cell count (SCC) as a standard method for detection of subclinical mastitis. One hundred random samples of cow's milk were collected from different dairy farms at Dakahlia Governorate, Egypt. SCC revealed that 27% of samples contained less than 2×10^5 SCC/ml. and were considered negative for presence of subclinical mastitis, while mastitic samples (73%) contained higher numbers of SCC exceeded 2×10^5 /ml. CMT results revealed that 27.0% of the examined cow's milk samples were negative and 73.0% were among the positive samples. The mean values of chlorine % of normal and mastitic cow's milk samples were 0.093 and 0.157, respectively. EC of normal and mastitic cow's milk samples revealed that the mean values were 4.08 and 7.42 mS/cm, respectively. Results showed significant correlation of these parameters in detection of subclinical mastitis milk samples.

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Introduction

During the last few decades, mastitis has become the most costly disease of dairy cows (Bennett *et al.*, 1999; Fourichon *et al.*, 2001), and it represents a food safety issue. Mastitis causes physical, chemical and bacteriological changes in milk and pathological changes in glandular tissue of udder (Sharma *et al.*, 2007). A primary stage of mastitis, subclinical mastitis, is an inflammation of the mammary gland without noticeable signs, although it is accompanied by 15-45% reduction in daily milk yield and altered milk composition (Swinkels *et al.*, 2005; Halasa *et al.*, 2007). In addition, it is considered a prevailing disease in dairy cows whereas every clinical case of mastitis, 15-40 subclinical cases occur (Kelly *et al.*, 2002).

Thus, early diagnosis of mastitis is important for reducing production losses and for enhancing the prospects of recovery. In addition, the identification of subclinically infected gland is urgently required for successful control of mastitis in dairy animals. While farmers can recognize clinical mastitis, subclinical mastitis can only be discovered by detecting of an inflammatory components and pathogens in the milk (Nielen *et al.*, 1995).

Inflammation of mammary gland is directly accompanied by an increase of somatic cell count (SCC) in milk (Rodriguez *et al.*, 2000). Therefore, many reports have considered SCC as a significant

marker for subclinical mastitis (Dürr *et al.*, 2008). Another test for detection of subclinical mastitis, California Mastitis test (CMT) has been accepted as a quick and simple test to predict SCC from individual quarters or bulk milk. While, an increase in CMT score corresponds to the increase in SCC, it is uncertain whether CMT or SCC scores can accurately reflect intramammary infection due to specific pathogens.

Electrical conductivity (EC) is now employed as a routine test for subclinical mastitis detection (Milner *et al.*, 1996). EC is influenced by sodium, potassium, calcium, magnesium, chlorine and other ions. EC of the milk increases due to an increased concentration of Na^+ and Cl^- . However, factors other than mastitis, like breed, lactation stage, milking interval and milk composition may affect milk EC. Moreover, many dairy producers especially those who still adopt hand milking, may not depend on EC as a routinely test for detection of subclinical mastitis. Due to the aforementioned economic and public health importance, the purpose of the present investigation is directed initially for detection of subclinical mastitis using SCC, CMT, WST, chlorine and EC and to compare the significance of each test against the standard SCC.

Materials and Methods

Samples collection

A total of one hundred raw milk samples were

*Corresponding author.
Email: drsalah_aal@yahoo.com

obtained as recommended by Mason (2006) and Wenz *et al.* (2006). Teats were efficiently cleaned and first streams of the milk were discarded. Any case that confirmed with udder inflammation or abnormal milk was completely excluded from this study (Radostitis and Blood, 1994).

Preparation of samples

Each sample was mixed thoroughly before being used for detection of mastitis (APHA, 1992).

Incidence of subclinical mastitis

California mastitis test (CMT)

About 2 ml of milk samples was squirted into a black plastic cup. Then, equal volumes of CMT reagent was added (Alkyl Aryl sulphonate). Cup was horizontally swirled in a circular motion gently. Result of the test was recorded immediately after 10 seconds (Schalm and Noorlander, 1957).

Somatic cell count (SCC)

Milk samples were examined automatically for Somatic cell count using Somatic cell counter. The sample was warmed in water bath at 35°C for 5 minutes, and then mixed automatically before reading (Radostitis *et al.*, 2000).

White side test (WST)

Five drops of milk were added to two drops of NaOH 4% on clean glass plate placed on dark black ground and mixed well and the reaction was graded according to the Scandinavian recommendations (Schalm *et al.*, 1971; Klastrup and Schmidt, 1974).

Chlorine test

Chlorine content in raw milk samples was determined following method described at Analysis of Milk and its Products, A lab manual (2005). In a porcelain dish, 10 ml of milk was added to 5 ml of nitric acid 25%, 5 ml of silver nitrate N/10 and 1 ml of ferric ammonium sulphate. Titration against ammonium thiocyanate N/10 was continued until a brown color appeared and remained for two minutes and R record (amount of ammonium thiocyanate N/10 used in titration). Appearance of a brown color which remains for two minutes indicates that chlorine content is 0.14% or more.

Measurement of electrical conductivity

Milk samples were examined automatically with the Afikim computerized milking and management system according to KAŞIKÇI *et al.* (2012)

Statistical analysis

All data was analyzed using Statistical Packages for the Social Sciences (SPSS) (Foster, 2001).

Results and Discussion

Apart from clinical form, Subclinical mastitis is inflicting great economic losses (Dhakal *et al.*, 2007). This is mainly attributed to sever drop in milk production, decrease in milk quality, increased veterinary expenses due to excessive use of medications, increased labor costs and increased culling rate and decrease reproductive efficiency in high producing animals (Bansal *et al.*, 2004). In addition, potential health risks were also encountered as milk from affected animals may harbor pathogenic organisms to human (Bilal *et al.*, 2004), increased risk of residues in the milk and consequently the possibility of public health hazards (Beck *et al.*, 1992).

SCC is positively correlated with the udder inflammatory condition. When udder is healthy, SCC in milk usually counted between 50,000 and 100,000 cells/ml and in some cases up to 200,000 cells/ml. If the SCC exceeded 200,000 cells/ml., animal is considered affected with subclinical mastitis (Skrzypek *et al.*, 2004). High SCC in milk reduces the quality of milk and inturn, dairy products, and affects milk shelf life and flavor, as well as cheese and butter fat yield.

Minimum SCC of normal cow's milk samples was 4.80×10^4 , while the maximum was 1.87×10^5 with a mean count of 1.02×10^5 . While the SCC positive samples showed a maximum 6.10×10^6 (table 1). Frequency distribution analysis revealed that 27% of samples lied at ranges of $<200 \times 10^3$ (normal samples). Other ranges of SCC had different samples' percentages (Figure 1). Higher values of SCC/ml. for mastitis milk samples were recorded by Elango *et al.* (2010), while lower figures were recorded by Bhutto *et al.* (2012). Reneau (1986) clearly stated that udder infection is the most important factor affecting SCC.

CMT principle is based upon the amount of cellular nuclear protein present in the milk sample, thus correlated to SCC (Greiner *et al.*, 2000). Thus, in relation to SCC, nearly similar outcomes were obtained using CMT. According to CMT, samples were divided into CMT Negative and positive. The samples that were considered negative using SCC (27), were also found negative using CMT. Regarding CMT scoring (date not shown), the highest incidence was recorded in CMT (+++) as (44.4%) and the lowest in CMT (+) as (23.8%) while CMT (++) was

Table 1. Statistical analytical results of SCC/ml. in examined cow's milk samples

Examined cow milk samples	No.	Minimum	Maximum	Mean	± S.E.M
Normal	27	4.80×10^4	1.87×10^5	1.02×10^5	9.73×10^3
Mastitis	73	4.30×10^5	6.10×10^6	3.90×10^6	1.80×10^5

SCC; somatic cell count.
S.E.M.: Standard Error of Mean.

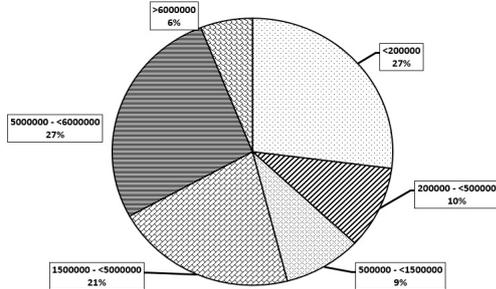


Figure 1. Frequency distribution of SCC/ml in examined cow's milk samples

(31.8%). Karimuribo *et al.* (2006) reported nearly similar findings. Lower results were reported by previous studies (Bhutto *et al.*, 2012; Jin-bo *et al.*, 2012).

CMT possesses many advantages, as the higher sensitivity, simplicity and accuracy. In addition, presence of foreign material, such as hair or other matter, does not interfere with the test. Although, as disadvantages for CMT; Scoring the test depends on individual testers variation, scores correlation to a nearly wide range of SCC, false positive reactions occur at early or late lactation, in addition to acute mastitis cases may not reacted positively due to the destruction of leucocytes by microbial toxins (Rice, 1981).

CMT was used by many researchers for detection of subclinical mastitis in cattle (Eshak, 2002; Aly, 2006; Joshi). Gokhale (2006) mentioned that CMT is the most sensitive (95.16%) and specific (98.02%) test for detection of subclinical mastitis. Iqbal *et al.* (2006) also confirmed the great sensitivity of CMT applied at farm or laboratory for detection of subclinical mastitis.

White side test (WST) is another test that still applied at low income economic countries due to its inexpensive nature. Using WST (data not shown), 25.0% were found negative and 75.0% were found positive among the 100 examined cow's milk samples. Ali *et al.* (2011) recorded lower results, as they found that overall prevalence of subclinical mastitis using WST was 44%. Also, Iqbal *et al.* (2004); Pitkala *et al.* (2004) and Muhammed *et al.* (2010) recorded an incidence of 54.37, 54.7 and 92%, respectively. These prevalence's variations might be attributed to regions, managements and scoring method (Ali *et al.*, 2011). However, Iqbal *et al.* (2004) reported the insensitivity of WST in detecting subclinical mastitis cases whereas WST failed to adequately correlate

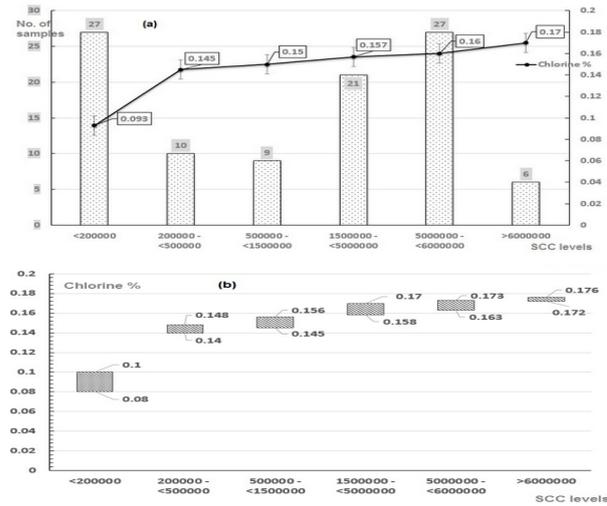


Figure 2. Statistical (a) mean values ± SE and (b) minimum and maximum values of chlorine % distributed according to SCC.

with SCC in detection of subclinical mastitic milk samples.

Regarding chlorine %, the minimum percentage of examined samples was 0.08 and this was recorded at samples which counted less than 2×10^5 of SCC/ml, while the maximum chlorine % was 0.176 and it was recorded at sample with SCC of more than 600×10^4 /ml. Based on SCC distribution, initial sharp increase in the mean of chlorine % was encountered between the first group of samples with SCC of less than 2×10^5 /ml and the second group of SCC till 5×10^5 /ml, then gradual increase was reported till the last group of SCC more than 6×10^6 /ml (figure 2 a & b). Normal chlorine percentage range of normal milk was defined by Elango *et al.* (2010) to be 0.08 to 0.14 %. Higher values of chlorine content for mastitis milk samples were recorded by Elango *et al.* (2010) while lower values were reported by Sharma *et al.* (2011).

Ductal and secretory epithelium malfunction due to microbial infection leads to sharp increase of sodium and chlorine, in addition to the breakdown of junctions between secretory cells, and the increased permeability of the blood capillaries. Thus, chlorine flowed into milk (Batavani *et al.*, 2007). This explaining the possibility of adopting chlorine percentage in milk as an indicator for presence of subclinical mastitis (Morsi *et al.*, 2000), however, chlorine % alone cannot judge the presence of mastitis as it usually give high results in colostrum or at late stage of lactation.

Depending on EC, samples were classified into normal (27 samples with EC values of less than 5 mS/cm) and mastitic (73 samples with EC of equal to 5 mS/cm or more). The minimum EC in examined normal milk samples was 4 mS/cm; the maximum was 4.3 mS/cm with a mean value of 4.08 mS/cm

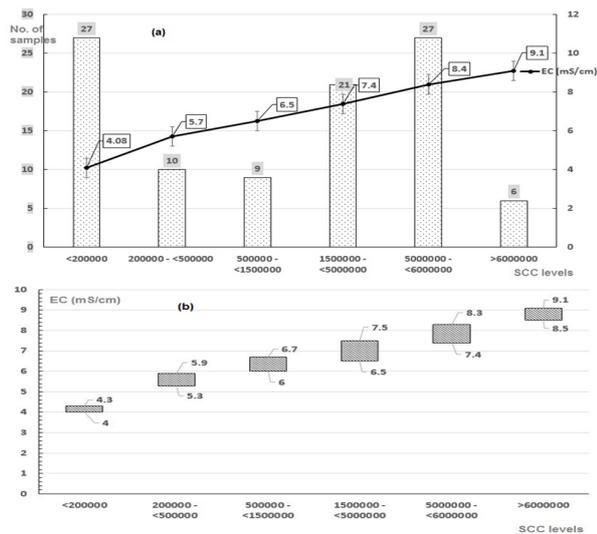


Figure 3. Statistical (a) mean values \pm SE and (b) minimum and maximum values of EC (mS/cm) distributed according to SCC

(figure 3a). Nearly similar findings were reported by Špakauskas *et al.* (2006) and El-barawy and Ali (2011). Higher findings were reported by Mansell and Seguya (2003). While in mastitic milk samples, the minimum was 5.30 mS/cm and the maximum was 9.10 mS/cm (figure 3b). Nearly similar findings were reported by Špakauskas *et al.* (2006) and Cavero *et al.* (2007). Higher findings were reported by Janzekovic *et al.* (2009), while lower values were recorded by Mansell and Seguya (2003). Simulating the figure of chlorine percentage's increase among SCC distribution groups, EC figure is nearly correlated to chlorine % (Figure 3a). This similarity is attributed to the sole dependence of EC on chlorine ions present in milk.

Milk EC is influenced by the health status of the udder. The ability of EC to differentiate cases with subclinical infection from healthy ones is not precise enough (Milner *et al.*, 1996). Although EC can represent a possible indicator, genetic factors may have a role in governing EC of milk (Ilie *et al.*, 2010). Grennstam (2005) recorded that if the EC value of a quarter is over 15% higher than the average of the two quarters with the lowest EC value, the quarter is considered as mastitis infected.

Statistically correlating achieved results, we could conclude that all indirect tests for screening of subclinical mastitis correlated significantly (with variable degrees) to each other's (table 2). While to SCC, EC; chlorine % and CMT have a high significant correlation coefficient ($p < 0.001$), WST had a significant one ($P < 0.01$).

Conclusion

The present study was designed to investigate subclinical mastitis in Egyptian dairy cows with

special emphasis to find a practical marker for its early diagnosis. In conclusion, screening tests should be applied by specialists to control mastitis and to have a proper correlation between the screening tests, more than one test should be recommended for detection of mastitis. SCC, CMT and EC findings represent valuable diagnostic methods in detection of cows with secretion disorder whose show no clinical signs of disease.

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