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Somaticell® as a screening method for somatic cell count from bovine milk

Avaliação do Somaticell® como método de triagem para contagem de células somáticas do leite de bovinos

Hélio Langoni[I*] Daniel da Silva Penachio[I] Diego Borin Nóbrega[I] Felipe de Freitas Guimarães[I] Simone Baldini Lucheis[II]

ABSTRACT

The objectives of the present study were to evaluate the correlation between electronic somatic cell count (eSCC) and Somaticell® under different milk somatic cell count (SCC) conditions and to different mastitis pathogens and calculate the, sensitivity, specificity and predictive values of Somaticell® using different SCC limits established by different countries. Three-hundred and forty milk samples were aseptically collected according to the California Mastitis Test (CMT) result. The Somaticell® and eSCC were carried out in all milk samples. The correlation between Somaticell® test results and electronic counts was determined according to the CMT, isolated pathogen and eSCC score. According to the SCC scores established, 26.5% milk samples showed score 1 (69-166x10³cells mL⁻¹), 26.8% score 2 (167-418x10³cells mL⁻¹), 27.4% score 3 (419-760x10³cells mL⁻¹) and 19.4% score 4 (761 to 1,970x10³cells mL⁻¹). According to Spearmann correlation test, eSCC and Somaticell® had a positive correlation (P<0.05) in almost all conditions (except eSCC score 2 and score 3). The r value obtained between the SCC and Somaticell® was 0.32. It was observed that as the SCC thresholds increased, the sensitivity values decrease and specificity increased. The predictive values remained constant among all limits. When the SCC limit is lower (<760,000cells mL⁻¹), Somaticell® resulted in higher counts than the SCC. As for samples with high SCC, Somaticell® resulted in lower counts than the eSCC. The correlation between the two methods remained relatively constant in all conditions and the sensitivity and specificity of the test is highly dependent of the threshold established. The results of this study suggest that Somaticell® is not useful to evaluate milk SCC, as its results are significant different from the eSCC. Therefore it could be used as a screening method, such as CMT, to detect an increase in the milk SCC.

Key words: *evaluation, somatic cell count, Somaticell®.*

RESUMO

Os objetivos do presente estudo foram avaliar a correlação entre a contagem eletrônica de células somáticas (eCCS) com o Somaticell® sob diferentes níveis de contagem de células somáticas (CCS) do leite e patógenos causadores de mastites, além de calcular a sensibilidade, especificidade e valores preditivos do Somaticell® utilizando diferentes limites de CCS estabelecidos pelos diferentes países. Trezentos e quarenta amostras de leite foram coletadas assepticamente após realização do California Mastitis Test (CMT). O Somaticell® e a eCCS foram realizados em todas as amostras de leite. A correlação entre o Somaticell® e a contagem eletrônica foi determinada de acordo com o CMT, patógeno isolado e escore de eCCS. De acordo com os escores de CCS estabelecidos, 26,5% das amostras de leite apresentaram escore 1 (69-166 x10³células mL⁻¹), 26,8% escore 2 (167-418x10³células mL⁻¹), 27,4% escore 3 (419-760x10³células mL⁻¹) e 19,4% escore 4 (761 to 1970x10³células mL⁻¹). A eCCS e o Somaticell® apresentaram correlação positiva em quase todos os escores estudados (exceto escore 2 e 3). O valor de r obtido entre CCS e o Somaticell® foi de 0,32. Observou-se que, quando o limite de CCS estabelecido aumentou, a sensibilidade decresceu e os valores de especificidade aumentaram. Os valores preditivos apresentaram-se constantes em todos os limites. Quando o limite de CCS era baixo (<760,000 células mL⁻¹), Somaticell® forneceu resultados consistentemente mais elevados que os valores de CCS. Já para amostras com CCS elevada, Somaticell® resultou em menores contagens que a eCCS. A correlação entre os dois métodos permaneceu relativamente constante em todas as condições e os valores de sensibilidade e especificidade do teste são altamente dependentes do limite estabelecido. Os resultados deste trabalho sugerem que o Somaticell® não é útil para avaliar a CCS do leite, pois seus resultados são significativamente diferentes da eCCS, no entanto, pode ser utilizado como método de triagem, tal como o CMT, para a detecção do aumento da CCS do leite.

Palavras-chave: *avaliação, contagem de células somáticas, Somaticell®.*

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INTRODUCTION

Somatic cell count (SCC) is a measure of the concentration of epithelial cells and leukocyte in milk that have been directed by the body as a defense against bacterial invasion. Therefore, it could be viewed as indicators of a cow's udder health status (BERRY et al., 2007).

There is a great variability in the SCC among individual animals (BENNEDSGAARD et al., 2003). The most important cause of variation in milk SCC is the infection status and SCC higher than 200,000cells mL^{-1} is generally regarded as being suggestive of intramammary infection (BREEN et al., 2009).

SCC is considered an important indicator for the qualification of raw milk by the dairy industry. Therefore, dairy producers should make every effort to ensure that milk SCC produced by their herd is constantly at the lowest possible level and thus meets the qualification limits in force (JANOSI & BALTAY, 2004). In the United States, the Pasteurized Milk Ordinance requires grade A milk produced on individual farms to have a bulk tank SCC <750,000 cells mL^{-1}. Dairy processors perform the tests and provide state regulatory agencies with monthly bulk milk SCC values from individual dairies to enforce milk quality regulations (PANTOJA et al., 2009). In Brazil, the current limit of bulk tank SCC is 750,000 cells mL^{-1}, while the one adopted in the Europe Union is 400,000 cells mL^{-1}.

The electronic somatic cell count (eSCC) is an accurate and reliable test to estimate milk SCC. However, this test is impractical for on-farm use on most cases. A variety of indirect tests are available to overcome this situation. For many years, the California Mastitis Test (CMT) (SCHALM & NOORLANDER, 1957) has been used as an on-farm screening test for detection of subclinical mastitis. The CMT and eSCC are high dependent, and are important mastitis diagnosis tools (BARBOSA et al., 2002). A relatively high proportion of epithelial cells in the SCC may result in a higher CMT score (JANOSI & BALTAY, 2004). However, the relationship between results of eSCC and CMT is not straight forward because of variability in SCC values within each CMT score.

Somaticell® (Madasa, São Paulo, Brazil) is a quick method to estimate the milk SCC. It is a modified version of Wisconsin Mastitis Test., performed in few minutes and provides a quantitative outcome. Somaticell® was developed to directly yield results as an equivalent to eSCC. It can be used in dairy herds to detect subclinical mastitis cases in milk quality programs (MEDEIROS et al., 2008; RODRIGUES et al., 2009), and it is also recommended for use in the milk from bulk tanks to verify, as a preliminary test , if it meets the current limits established by the legislations.

Our hypothesis was that Somaticell® had a high correlation coefficient with eSCC independent of the mastitis pathogen and CMT score, and also could be used in bulk milk with different SCC limits. The objectives of the present study were to evaluate the correlation between eSCC and Somaticell® under different milk SCC conditions and to different mastitis pathogens. Additionally it was the objective to calculate the, sensitivity, specificity and predictive values of Somaticell® using different SCC limits established by different countries.

MATERIAL AND METHODS

Three-hundred and forty milk samples from 340 different cows from ten commercial herds located in São Paulo state were used in this study. One random teat per cow was chosen to be sampled. The milk samples were previously categorized according to the result of the California Mastitis Test (SCHALM & NOORLANDER, 1957), so that samples with various degrees of SCC were used. CMT reaction was visually scored in a four-point scale. Clinical mastitis cases were not sampled.

Duplicate milk samples were collected in sterile glass vials after teat ends were thoroughly scrubbed with a 5% alcohol iodine solution-soaked cotton pad (NMC, 1999). After discarding the first milks streams, 60mL of milk were collected and kept at 4°C until bacteriological assays and analyses of SCC. Around 5mL of milk were collected for microbiological exam, 5mL for Somaticell® test and 50mL were used to electronic determination of SCC.

The Somaticell® was carried out according to instructions of the manufacturer using the refrigerated samples collected within 5 hours. Test materials consist of a single-use plastic graduated vial with a predetermined scale of somatic cells, a perforated cap, a straw for mixing, and a reagent. The test procedures were as follows: 2mL of milk was added to the graduated vial followed by 2mL of reagent. The straw was used to mix the solution using 30 up-and down movements in a 20-seconds interval. The cap was then used to close the vial, and the vial was inverted for 30 seconds to allow noncoagulated solution to drain from the vial. The vial was then returned to the upright position, and after 5 seconds, the value indicated on the vial SCC equivalent scale (Somaticell® has 41 outcomes for milk SCC; its maximum and minimum values are 1,970,000cells mL^{-1} and 69,000cells mL^{-1} respectively) was reported. The eSCC was performed using a previously calibrated Somacount 300 (Bentley Instruments Inc, Chaska, MI)

Microbiological culture was done in duplicate according to standard procedures of the National Mastitis Council (NMC, 1999). Ten microliters of milk was

inoculated onto blood agar and MacConkey agar to enhance the detection of Enterobacteriaceae. Plates were incubated at 37°C and read at 24, 48, and 72h. Organisms were identified by gross colony morphology and Gram stain and by further confirmatory techniques as necessary. *Staphylococci* were subjected to coagulase test, and grouped as coagulase-positive ***Staphylococcus*** (CPS) and coagulase-negative ***Staphylococcus*** (CNS). Gram-positive, catalase-negative cocci were identified as belonging to the ***Streptococcaceae*** family and subjected biochemical tests according to QUINN et al. (2005). The gram-positive, pleomorphic and catalase-positive coccobacilli were classified within the ***Corynebacterium*** genus. Gram negative bacilli were streaked on triple sugar iron agar (TSI) and subjected to the citrate, motility, indole production and gas production tests. The presence of >3 bacterial species was considered a contaminated sample. Major mastitis pathogens included isolation of CPS, ***Streptococcus*** spp., or coliform species, while minor mastitis pathogens were defined as CNS or ***Corynebacterium*** species.

An intramammary infection (IMI) was defined as the presence of 3 or more colonies of the same type (NMC, 1999). If one of the duplicate samples was contaminated, the results from the uncontaminated duplicate alone were used to diagnose infection. When a quarter had both a major and a minor pathogen isolated at the same time, the quarter was defined as infected with the major pathogen for that sampling occasion. Milk samples that were either contaminated or not collected were assigned as missing values for analysis.

Statistical analyses

Univariate analysis was performed to determine measures of central tendency, dispersion, and distribution characteristics of Somaticell® and eSCC under different conditions (presence/absence of IMI, CMT score, pathogen type). The data normality was checked by Shapiro-Wilk test. Somatic cell counts were log-transformed (base 10) to achieve homogenous variance.

The correlation between Somaticell® test results and electronic counts was determined using Spearmann non-parametric test. For all correlation tests, the eSCC values that were outside Somaticell® range were adjusted to the respective limits. To descriptive statistics, eSCC values were used with no correction. The correlation was calculated under different circumstances: the presence/absence of IMI, CMT score, eSCC scores (score 1=69-166x10^3cells mL^{-1}, score 2=167-418x10^3cells mL^{-1}, score 3=419-760x10^3cells mL^{-1} and score 4=761 to 1,970x10^3cells mL^{-1}) and type of pathogen isolated.

Linear regression using least square methods was applied to estimate the exact value of eSCC using the Somaticell® result. In this analysis, only samples with eSCC values from 69,000 to 1,970,000 cells mL^{-1} were included. Both eSCC and Somaticell® were log transformed. Forced entry method was used. Durbin-Watson statistics test was used to verify the assumption of independent errors. Regression coefficients were calculated as the 95% confidence interval, *t* statistic value and its significance, and R^2 value (FIELD & MILES, 2010).

To test Somaticell® sensitivity, specificity and predictive values three SCC thresholds were evaluated based on limits established by different countries. The eSCC limits were adjusted to analyze the data, fitting it to one common value for both Somaticell® and eSCC. We adopted the following gold standards: 418,000 somatic cells mL^{-1}, 500,000 somatic cells mL^{-1} and 760,000 somatic cells mL^{-1} Still, the closest Somaticell® reading to the standard threshold of subclinical mastitis (200,000 cells mL^{-1}) was 205,000 cells mL^{-1}. Therefore, 205,000 cells mL^{-1} was used as the threshold to define subclinical mastitis for both methods of SCC determination. The correlation between the Somaticell® result and the CMT was also performed using the Spearmann correlation coefficent.

The level of statistical significance was set at 0.05 for all analyses. The analyses were performed with the SAS 9.2 software (SAS, 2008).

RESULTS

Three-hundred and forty teats from 340 different cows were used in this study. Regarding the CMT results, 8.2% milk samples presented a negative result (score 0), 37.6% score one, 34.1% score two and 15.3% presented the score three.

Mean and median values for eSCC and Somaticell® under different conditions are showed in table 1. Somaticell® and eSSC showed similar results is several conditions. The mean value of cellularity in eSCC was 504.15x10^3 cells mL^{-1}, and in Somaticell® was 549.16x10^3 cells mL^{-1}. The majority of milk samples presented low SCC in the eSCC; 64.4% of samples were in the range of 69-166x10^3 cells mL^{-1} (score 1), 7.6% of samples in the 167-418x10^3 cells mL^{-1} range (score 2), 10.9% in the range of 419-760x10^3 cells mL^{-1} (score 3), and 17.1% were between 761 and 1,970x10^3 cells mL^{-1} (score 4). Still according to the SCC scores established, Somaticell® presented a different distribution of results: 26.5% milk samples showed score 1 (69-166x10^3 cells mL^{-1}), 26.8% score 2 (167-418 x10^3 cells mL^{-1}), 27.4% score 3 (419-760x10^3 cells mL^{-1}) and 19.4% score 4 (761-1,970x10^3 cells mL^{-1}).

In three scores of cellularity (score 1, score 2 and score 4), the two methods showed distinct results as shown in table 1 (score 1=77.59 and 381.11x10^3 cells mL^{-1} for eSCC and Somaticell® respectively, score 2=311.10

Table 1 - Mean, median, 25 (P25) and 75 percentiles (P75) values and result of the Spearmann correlation test between the electronic somatic cell count (eSCCx10^3 cells mL^{-1}) and Somaticell® (x10^3 cells mL^{-1}) using all samples (total), according to presence/absence of intramammary infection (IMI), result of the California Mastitis Test (CMT), type of isolated pathogen (major or minor), and score of electronic somatic cell count (1=69-166; 2=167-418; 3=419-760; 4=761-1,970x10^3 cells mL^{-1}).

	Condition	Mean	Median	P25	P75
eSCC	Total[a]	504.15	59	11.25	522
	Absence of IMI[b]	436.88	60	8	414
	Presence of IMI[c]	536	52	12	536
	CMT 0[d*]	194.94	59.5	3	154.25
	CMT 1[e]	247.16	18.5	7	321
	CMT 2[f]	681.07	79	19.5	653.75
	CMT 3[g]	907.52	170.5	39.75	1,027.75
	Major pathogens[h]	678.52	55.5	11	519
	Minor pathogens[i]	483.55	51	15.5	597.75
	Score 1[j*]	77.59	69	69	69
	Score 2	311.1	322	237.25	386.75
	Score 3	621.02	608.5	535.25	701
	Score 4[k]	1,601.34	1,668.00	1,247.00	1,970.00
Somaticell®	Total[a]	549.16	379	166	630
	Absence of IMI[b]	440.33	244	98	500
	Presence of IMI[c]	598.43	457	224	760
	CMT 0[d*]	164.78	98	91.25	166.5
	CMT 1[e]	340.86	263	127	470
	CMT 2[f]	650.06	500	291.75	760
	CMT 3[g]	1,043.63	875	537.5	1,800.00
	Major pathogens[h]	866.27	630	205	630
	Minor pathogens[i]	499.84	379	349.75	1,615.00
	Score 1[j*]	381.11	282	108	500
	Score 2	651.43	471	229.5	960
	Score 3	648.14	500	340	630
	Score 4[k]	1,097.78	875	560	1,970.00

[a-k] Values of condition followed by the same letter shows a significant Spearmann correlation coefficient (P<0.001, one-tailed) between the eSCC and Somaticell® at this condition.
[*] P< 0.05 (one-tailed).

and 651.43x10^3 cells mL^{-1} and score 4=1,601.34 and 1,097.78x10^3 cells mL^{-1} for the two methods). Samples containing major pathogens, presence of IMI, and with CMT score 3, presented higher results for both methods than samples with minor pathogens, absence of IMI and CMT score 0, 1 and/or 2 respectively.

The most common isolated pathogens were ***Corynebacterium bovis*** (26.7%), coagulase-negative ***Staphylococcus*** (22.7%), ***Streptococcus dysgalactiae*** (7.6%), ***Streptococcus uberis*** (3.9%), CPS (3.9%), ***Escherichia coli*** (2.1%) and ***Klebsiella pneumoniae*** (0.6%). In 32.4% of samples, no growth was observed in the microbiological exam. Regarding the pathogens type, 73.1% of isolated pathogens were classified as minor and 26.9% classified as major pathogens.

In each condition (CMT results, scores of cellularity, major/minor pathogens and presence/absence of IMI), Spearmann correlation test among the two methods was applied and its *P* significance value obtained (Table 1). The correlation between the two tests, independent of the condition was considered average (0.32). In two conditions (score 2 and 3) it wasn't observed a significant correlation between the two methods.

Using the scatter plot, it was suspected that the two variables had a linear relationship. The linear regression resulted in adjusted R^2 value equals to 0.047, β_0=1.942 (1.497 e 2.388 as lower and upper bounds to 95% confidence intervals), X_1=0.233 (standardized regression coefficient) with 0.070 and 0.403 as limits to the 95% confidence interval.

According to the thresholds established by the countries, 71.5% of samples presented SCC between 0-400 x10^3 cells mL^{-1}, 74.1% counts between 0-500x10^3 cells mL^{-1} and 82.9% counts between 0-750x10^3 cells mL^{-1}. The

Somaticell® results when adopting the same limits were 50.6% of samples in the range of 0-400x10^3 cells mL^{-1}, 60.6% in the 0-500x10^3 cells mL^{-1} interval and 78.5% samples with counts between 0-750x10^3 cells mL^{-1}.

Sensitivity, specificity and predictive values according to the SCC limits established are presented in table 2. The correlation coefficient between Somaticell® and the CMT was 0.474, (P<0.001).

DISCUSSION

In the present study, minor contagious pathogens like ***Corynebacterium bovis*** and CNS were responsible for the most subclinical mastitis cases. CNS have been frequently isolated from bovine mastitis cases (SCHREINER & RUEGG, 2003). Environmental streptococci were isolated in 11.5% of milk samples, and were the most isolated environmental pathogens.

Somatic cell counts are a useful measure of udder health (GREEN et al., 2004). Studies indicate that different pathogens are associated with different magnitude of SCC increase (DE HAAS et al., 2002; SCHREINER & RUEGG, 2003). In the present study, higher levels of SCC was observed for major pathogens (mean value of 678.52 cells mL^{-1}) in contrast to lower levels for minor pathogens (mean value of 483.55 cells mL^{-1}).

In our study, the r value obtained between the eSCC and Somaticell® was 0.32, in contrast to 0.92 observed by RODRIGUES et al. (2009). In that occasion, the majority of milk samples presented low counts (around 200,000 cells mL^{-1}), which was not the case in our study where the majority of samples consisted of high SCC milk. This result suggests that Somaticell® may show a better performance in low SCC.

eSCC and Somaticell® had a positive correlation (P<0.05) in almost all studied conditions (different scores of SCC, CMT and mastitis pathogens). To eSCC above 200,000 cells mL^{-1} and bellow 1,000,000 cells mL^{-1}, demonstrated mainly by score two and score three, no correlation could be detected, showing a possible failure in Somaticell® to estimate the SCC in this range.

According to the linear regression, the alteration in one unit of Somaticell® leads to a change in 0.233 units in eSCC, added the value of 1.942. Somaticell® is a discrete numerical variable, which is not the same situation of eSCC. Still, the absence of a significant correlation in scores two and three can interfere with the linear regression. In conclusion, is not recommended to estimate the exact count of somatic cells based on the outcome of Somaticell®.

Sensitivity estimates the probability of the test to be positive when there is an increase in eSCC. In table 2, it is observed that as the SCC thresholds increased, the sensitivity values decrease and specificity increased. The predictive values remained constant along all limits

When higher SCC thresholds were established, the number of false negative samples was increased. Values of P25 for the score 4 of Somaticell® were lower than those obtained for eSCC (Table 1). The Somaticell® provided low results for samples with high counts, which resulted in a drop in test sensitivity under high SCC limits. When the SCC limit was lower (<760,000 cell mL^{-1}), Somaticell® gave results consistently higher than the eSCC values, as indicated in table 1 (score 1 values are consistently higher for Somaticell® in relation to eSCC). This directly reflects on the specificity of the test, which increased as the threshold was higher. With low thresholds, the result of Somaticell® was consistently higher, generating false-positive results and decreasing the specificity, but with a high sensitivity as its outcome is usually above the true SCC value. From some value between score 3 and score 4 of SCC, samples with high eSCC provided lower results in Somaticell®, and a high result in Somaticell® probably reflects a high count sample (high specificity).

A major utility for a rapid SCC test that presents quantitative outcomes would be during the milk's arrival at the dairy plant reception platform., although not this was not availed in the present study. High SCC milk could be detected and could have a different destination than the high quality product. Nowadays,

Table 2 - Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in percentiles of Somaticell® adopting different electronic somatic cell counts (eSCC) limits as the gold standards.

	eSCC limits (x10^3 cells mL^{-1})			
	205	418	500	760
Sensitivity	90.59%	81.40%	70.45%	60.34%
Specificity	39.01%	63.40%	71.43%	86.52%
PPV	43.80%	47.02%	46.27%	47.95%
NPV	88.77%	89.53%	87.38%	91.39%

the CMT is still on use in Brazil in some milk plataforms, and may lack on standardization due to its subjective interpretation. Somaticell® could prove to be an important tool to be used on these situations.

High counts in Somaticell® could only be trusted if the threshold adopted by the country is high (for example Brazil, which adopts currently the threshold of 750,000 cells mL^{-1}) due mainly to the high specificity of the test under these conditions (although the test in not the gold standard method to count milk somatic cells). A sample that resulted in a count of 960,000 cells mL^{-1} in Somaticell®, for example, would present about 86% chance of really having an electronic count greater than 760,000 cells mL^{-1}, which is relatively reliable. In these situations, Somaticell® could be used in dairy industries as a screening test to avoid contamination of the high quality milk with a product of lower quality. However, low results in Somaticell® would not discard the probability that this product is in fact of low quality, since the sensitivity of the test is not appropriate. A milk sample with a result of 630,000 somatic cells mL^{-1} in Somaticell®, has about 60% chance to actually contain a value less than 760,000 cells mL^{-1}. In contrast, the opposite situation is observed in countries with high SCC limits.

The threshold of 205,000 cell mL^{-1} was established as indicative of IMI. Similar values had already been used by other authors (BREEN et al., 2009). It is well established that cows with a SCC higher than 200,000 cells mL^{-1} are more likely to have an IMI than cows with a lower SCC (GREEN et al., 2007). In our study, we found out that Somaticell® had a high sensitivity (90.59%) and low specificity (39.01%) for the threshold of 205,000 cells mL^{-1}, which indicates that it is a useful tool for detecting an increase in SCC, but possibly presenting some false-positives results. Nevertheless, it could be used as a screening method as the CMT is used. The correlation coefficient between the two methods was considered moderate (FIELD & MILES, 2010) which justifies the use of one or another. The fact that the Somaticell® results in a quantitative outcome, could prevent any subjective interpretation of its results.

CONCLUSION

Electronic somatic cell count and Somaticell® had a significant correlation in all conditions, except in mild SCC samples represented mainly by SCC score 2 (167-418x10^3 cells mL^{-1}) and score 3 (419-760x10^3 cells mL^{-1}), being, therefore, not recommended to evaluate the milk SCC, as in this interval is the most important values of SCC established in quality programs. It is not recommended to estimate the exact SCC based on the outcome of Somaticell®. A drop in the test sensitivity is observed under high SCC limits. In contrast, it was observed low specificity values under low SCC limits. Still, it could be considered a screening method to detect an increase in milk SCC, like the CMT.

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