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Short communication: Evaluation of an on-farm test to estimate somatic cell count

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ABSTRACT

The objective of this study was to compare the results of an on-farm test, named Somaticell, with results of electronic cell counting and for milk somatic cell count (SCC) among readers. The Somaticell test correctly determined the SCC in fresh quarter milk samples. Correlation between Somaticell and electronic enumeration of somatic cells was 0.92 and κ coefficient 0.82. Using a threshold of 205,000 cells/mL, the sensitivity and specificity for determination of intramammary infections were 91.3 and 96.0%, respectively. The SCC was greater for milk samples from which major mastitis pathogens were recovered. Minor variation among readers was observed and most likely associated with the mixing procedure. However, the final analysis indicated that this variation was not significant and did not affect the amount of samples classified as having subclinical mastitis. The on-farm test evaluated in this study showed adequate capacity of determining SCC on quarter milk samples and may be considered as an alternative for on-farm detection of subclinical mastitis.
Key words: somatic cell count, on-farm test, subclinical mastitis

Determination of udder infection status has traditionally involved the use of standard laboratory tests such as microbiological culture and SCC (Ruegg, 2003). Although these tests are reliable and accurate, they are impractical for on-farm use, and alternatives have been developed. A variety of indirect tests are available for determination of IMI (Read et al., 1969). For many years, the California Mastitis Test (**CMT**) has been used as an on-farm screening test for detection of subclinical mastitis. However, the relationship between results of electronic SCC and CMT is not straight forward because of variability in SCC values within each CMT score (Ruegg, 2003). To overcome this situation, indirect on-farm tests that produce a numeric value for SCC have been created (Barratt et al., 2003; Ruegg et al., 2005; Moon et al., 2007). The PortaSCC (PortaScience, Portland, OR) is a qualitative test that uses an algorithm to convert results of an enzymatic reaction into an estimated SCC; the test procedure requires a 45-min incubation period. The Direct Cell Counter (DeLaval, Tumba, Sweden) and C-Reader system (Digital Bio Technology Co., Seoul, Korea) are new technologies that utilize electronic counting of somatic cells. Somaticell (Madasa, Sao Paulo, Brazil) is a modified Wisconsin Mastitis Test (Thompson and Postle, 1964) that is performed in few minutes and results in a quantitative outcome. Somaticell uses the same methodology as Wisconsin Mastitis Test but was developed to directly yield results as an equivalent SCC. To achieve this characteristic, a new SCC scale was determined for the test vial using milk samples in which actual electronic SCC were previously measured (Machado et al., 2003). The objectives of this study were to evaluate test characteristics of the Somaticell test for determination of SCC and IMI, and to verify test variability among readers and duplicate milk samples.

Milk samples were obtained from cows located on 2 commercial dairy herds in Wisconsin. Samples from the first herd (n = 300) were collected randomly by sampling one-quarter of every other cow at milking time. To increase the number of samples with high SCC, samples from the second herd (n = 25) were chosen from cows that had SCC >400,000 cells/mL on the last DHI test, that had been performed 1 wk before sampling. In this case, milk samples were collected randomly from one-quarter of each high-SCC cow. Only milk samples with no visual abnormalities were used. Samples were collected from a single mammary quarter per cow to avoid the effect of quarter interdependence (Barkema et al., 1997) and to simplify statistical analysis. After preparation of the udder for milking, teat ends were cleansed using aseptic procedures (National Mastitis Council, 1999), and 70 mL of milk was collected. Milk samples were collected by study personnel and stored in a cooler for transport to the laboratory. Two milk samples were lost during transport due to leakage. At the laboratory, each sample was homogenized and split in 3 parts. Fif-

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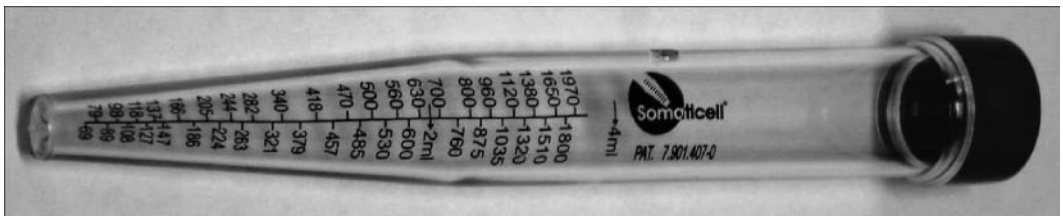

Figure 1. The test vial demonstrating graduation of SCC readings.

teen milliliters was saved to perform Somaticell test, 45 mL was sent for electronic determination of SCC, and 10 mL was kept for microbiological examination.

The Somaticell was carried out at the laboratory according to instructions of the manufacturer using the refrigerated samples collected within 5 h. Test materials consist of a single-use plastic graduated vial with a predetermined scale of somatic cells (Figure 1), a perforated cap, a straw for mixing, and a reagent. The test procedures were as follows: 2 mL of milk was added to the graduated vial followed by 2 mL of reagent. The straw was used to mix the solution using 30 up-and-down movements in a 20-s interval. The cap was then used to close the vial, and the vial was inverted for 30 s to allow noncoagulated solution to drain from the vial. The vial was then returned to the upright position, and after 5 s, the value indicated on the vial SCC equivalent scale was reported. One person was responsible for testing all 323 milk samples.

In a separate experiment, a subset of 100 quarter milk samples was used to allow 3 separate individuals to perform the test in duplicate. For this experiment, the sample population was composed of 83 samples with SCC <100,000 cells/mL, 10 samples with SCC of 100,000 to 500,000 cells/mL, and 7 samples with SCC >500,000 cells/mL. Each reader was asked to study the test manual before executing it, and communication among readers was not allowed. All tests were performed blindly in that readers were without knowledge of milk sample identity.

Electronic enumeration of somatic cells was performed using Fossomatic (Foss NIRSystems Inc., Laurel, MD). Microbiological culture was done in duplicate according to standard procedures of the National Mastitis Council (1999). One hundred microliters (0.10 mL) was adopted as the inoculum volume to enhance bacterial recovery (Dinsmore et al., 1992). Milk was streaked on one-half of a blood agar plate and MacConkey plate that were incubated at 37°C for 24 to 48 h. Morphology and hemolysis pattern of bacterial colonies obtained from plates with ≥3 cfu were determined, and organisms were differentiated by means of standard microbiologic methods. *Staphylococcus* was classified as *Staphylococcus aureus* and CNS based on mannitol fermentation and tube coagulase test. Microbiological detection limit was defined as ≥3 cfu of a particular organism and <3 colony types on the plate. Plates with >2 colony types were considered contaminated. Intramammary infection was defined as isolation of the same pathogen from both duplicate milk cultures.

Mastitis pathogens were grouped as major pathogen (*Staph. aureus*, environmental streptococci, coliforms) or minor pathogen (CNS, *Corynebacterium* spp.) for statistical analysis. Samples containing both types of pathogen were classified as major pathogens (n = 6). Samples considered contaminated (n = 9) were excluded from analysis.

Minimum and maximum Somaticell readings are equivalent to 69,000 cells/mL and 1,970,000 cells/mL, respectively (Figure 1). For comparing SCC between methods, results of electronic SCC that were outside of the Somaticell range were recorded as 69,000 cells/mL for lower results (n = 239) and as 1,970,000 cells/mL for higher results (n = 17). The closest Somaticell reading to the standard threshold of subclinical mastitis (200,000 cells/mL) is 205,000 cells/mL. Therefore, 205,000 cells/mL was used as the threshold to define subclinical mastitis for both methods of SCC determination. Somatic cell counts were log-transformed (base 10) to achieve homogenous variance.

The correlation between Somaticell test results and electronic counts was determined using Pearson coefficients, and the κ coefficient was calculated to determine agreement between methods. Sensitivity, specificity, positive predictive value, and negative predictive value were defined for Somaticell using electronic SCC as the gold standard. Sensitivity and specificity were also assessed for milk samples showing growth of mastitis major pathogens. The relationship between SCC and presence of pathogen in milk sample was estimated for both methods of SCC determination using a mixed linear model. Herd was included in the model as a fixed effect variable.

For the second experiment, results of Somaticell performed in duplicate by different readers were compared using Pearson correlation test and paired *t*-test. The

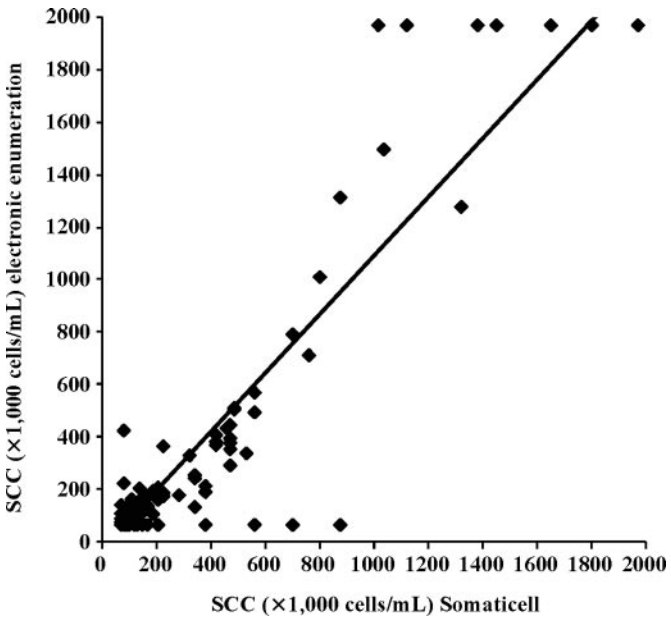

Figure 2. Correlation between Somaticell test (Madasa, Sao Paulo, Brazil) and electronic enumeration of somatic cells.

χ^2 test was performed to assess associations between reader and definition of subclinical mastitis. A mixed linear model was used to verify the relationship between SCC readings and duplicate, reader, and duplicate-reader interaction. Descriptive and statistical analyses were performed using SAS for Windows (SAS Institute, 2002–2003). Statistical significance was defined at $P \leq 0.05$.

The mean value of milk samples analyzed by the Somaticell test was equivalent to 214,000 cells/mL (median = 79,000), and the mean SCC of electronic count was 216,000 cells/mL (median = 69,000). The majority of milk samples presented low counts of somatic cells; the value of 69,000 cells/mL was registered 122 times for both methods. Correlation between Somaticell test results and electronic counts was 0.92 ($P < 0.001$; Figure 2). The same correlation was observed between the portable electronic counter Direct Cell Counter (**DCC**) and the standard electronic count (Ruegg et al., 2005). Another portable electronic counter, C-reader system, showed high correlation (0.93 to 0.96) with the standard electronic count testing raw milk and controlled samples (Moon et al., 2007).

The sensitivity of Somaticell (probability that Somaticell reading exceeded 205,000 cells/mL when gold standard, determined using electronic SCC, was greater than the threshold) was 91.3%, and specificity (probability that Somaticell reading did not exceed 205,000 cells/mL when the gold standard was less than the threshold) was 96.0%. Brito et al. (1997) used a threshold of 200,000 cells/mL and estimated that the specificity of CMT was about 95%. However, the sensitivity of CMT decreased as the CMT threshold was increased (79% for negative vs. trace, score 1, score 2, and score 3; 61% for negative and trace vs. score 1, score 2, and score 3; 34% for negative, trace, and score 1 vs. score 2, and score 3). In many circumstances, the greatest advantage of CMT use is when the test is read as negative or positive (trace, score 1, score 2, and score 3) to achieve better sensitivity. The Somaticell test may be useful in some circumstances because of the ability to derive quantitative estimates of the SCC.

The positive predictive value (probability that a sample having Somaticell over 205,000 cells/mL came from a quarter with SCC greater than the threshold, as determined using the gold standard) was 79.2%, and the negative predictive value (probability that a Somaticell reading under 205,000 cells/mL came from a quarter with SCC less than the threshold, as determined by the gold standard) was 98.5%. The predictive value of a test is influenced by disease prevalence in the studied population; a disease with greater prevalence increases the probability of its detection (Martin et al., 1987). In the present study, prevalence of subclinical mastitis was low, 83.6% of electronic SCC were below 205,000 cells/mL, and negatively affected the positive predictive value of Somaticell test. According to Ruegg (2003), in herds with low prevalence of subclinical mastitis, a good strategy to improve the positive predictive value of a diagnostic test is to increase the SCC threshold for infection definition. In the population of this study, it would be necessary to set the threshold at 600,000 cells/mL to increase the positive predictive value to approximately 90%.

The observed agreement between Somaticell test results and electronic counts to classify milk samples by infection status was 95.3%. The κ coefficient showed agreement between methods of 0.82 when the proportion of agreement by chance alone was excluded. According to Martin et al. (1987), a κ value >0.6 indicates a satisfactory amount of agreement. In this case, it is proper to say that Somaticell was able to correctly detect milk samples from healthy and infected mammary quarters. Similar agreement was seen by Ruegg et al. (2005) testing the portable electronic counter DCC. Using a threshold of 250,000 cells/mL to define subclinical infection, the DCC showed 95.6% of observed agreement with the standard electronic count and κ coefficient of 0.90.

Microbiological culture of milk samples yielded 5.9% with major pathogens, 8.1% with minor pathogens, 83.2% with negative results, and 2.8% with contaminated results. A significant relationship was found between culture results and SCC of both methods (P

< 0.001). Milk samples containing major pathogens had the greatest $\log_{10}$ of the Somaticell value (6.04) and $\log_{10}$ SCC of electronic count (6.13). The $\log_{10}$ in samples containing minor pathogens was moderate, 5.42 and 5.36 for Somaticell test and electronic count, respectively. On the contrary, samples that had negative results showed the lowest $\log_{10}$, 5.05 and 4.98 for Somaticell test and electronic count, respectively. The $\log_{10}$ of contaminated samples was equivalent to the $\log_{10}$ of negative samples.

The portable electronic counter DCC showed similar patterns between the $\log_{10}$ of SCC and culture results (Ruegg et al., 2005). The $\log_{10}$ of SCC obtained by the portable electronic counter DCC was greater in milk samples containing major pathogens (5.8) or minor pathogens (5.5) than in milk samples that were negative (5.1) or contaminated (5.0; $P < 0.001$). For this last study, milk samples also had few culture results yielding pathogens. Microbiological results were classified as major pathogen (*S. aureus*, environmental streptococci, *Escherichia coli*) 2.3%, minor pathogen (CNS, *Corynebacterium* spp.) 8.2%, negative samples 61.1%, and contaminated samples 28.4%.

The probability of Somaticell reading exceeded 205,000 cells/mL when the milk sample had growth of major mastitis pathogen, compared with growth of minor pathogen or no growth, was calculated to verify test usefulness in diagnosing main IMI. Sensitivity was 94.4%, and specificity (probability that Somaticell reading did not exceed 205,000 cells/mL when the milk sample did not show presence of major pathogen) was 88.4%. Only 1 sample of those having growth of major pathogens exhibited Somaticell reading below 205,000 cells/mL. Samples having growth of minor pathogens showed greater variability on Somaticell readings; around one-half displayed Somaticell readings below the threshold.

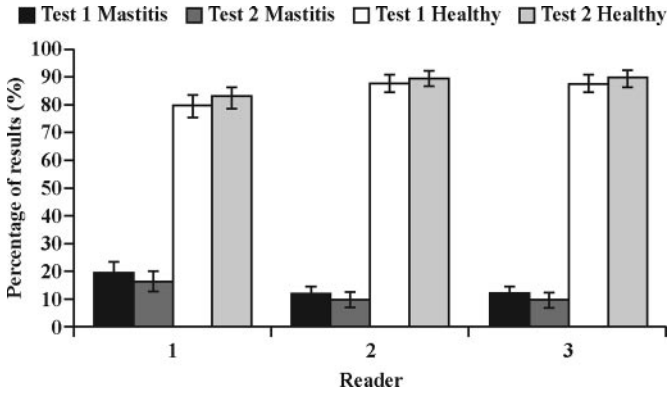

Figure 3. Results of Somaticell test (Madasa, Sao Paulo, Brazil) classified by subclinical mastitis per reader.

Della Libera et al. (2002) used the CMT and microbiological culture to classify mammary quarters as "clinically healthy" (CMT and culture-negative), "carrier" (CMT-negative and culture-positive), "mastitis without bacterial recovery" (CMT-positive and culture-negative), and "infectious mastitis" (CMT and culture-positive). When electronic SCC values were compared with CMT results, the SCC values differed for quarters classified as "clinically healthy" as compared with quarters classified as "carrier." The CMT test was unable to distinguish these differences in SCC due to insensitivity to small differences in cell numbers. For this reason, quantitative tests for SCC are more desirable in comparison to qualitative tests that present classes with variable ranges of SCC that most of the time overlap (Read et al., 1969).

Another advantage of having a numeric value for SCC was verified by Law (2004). This researcher proposed the use of a reflectometer to read the intensity of color generated by the qualitative test PortaSCC to get quantitative data from it. The PortaSCC readings based on visual interpretation of color intensity were compared with electronic counts and displayed correlation of 0.63, sensitivity of 76%, and specificity of 94% using a threshold of 200,000 cells/mL to define infection (Barratt et al., 2003). After associating the reflectometer to the test, correlation increased to 0.87, and sensitivity and specificity were 87 and 91%, respectively (Law, 2004). Later, Amaral and Ruegg (2005) worked with a meter developed for the PortaSCC test and also obtained adequate correlation for the test (0.81), comparing it to electronic counts. When a threshold of 200,000 cells/mL was used to define infection, there was 87.8% of observed agreement between methods, and κ coefficient was 0.73.

The outcomes of Somaticell test by duplicate and reader are shown in Table 1. The mean values, but not the median, recorded by reader 1 were greater than counts of reader 2 and 3. The correlation between duplicates within reader was significant and greater than 0.80. Correlation of reader 1 was approximately 0.13 correlation units less as compared with the other readers. The difference of $\log_{10}$ Somaticell values between duplicates within reader was equal and not significant for reader 2 and 3. A significant difference in $\log_{10}$ Somaticell values between duplicates was observed for reader 1. The procedure of test mixing, that involves a combination of time and movement (30 up-and-down movements in a 20-s interval), could be the major point of test variability that affected results of reader 1. The solution, milk plus reagent, that was not well mixed or was mixed during a different period of time, did not allow proper action of reagent originating incorrect reading. The SCC values of 180 milk samples read in

Table 1. Results of Somaticell test (Madasa, Sao Paulo, Brazil) performed in duplicate per reader

	SCC (×1,000 cells/mL)				Correlation, test 1 vs. 2[1]		Difference, test 1 vs. 2[2]	
	Test 1		Test 2					
Reader	Mean	Median	Mean	Median	r	*P*-value	Difference	*P*-value
1	194	79	179	69	0.82	<0.001	−0.02	0.041
2	153	69	144	79	0.95	<0.001	−0.01	0.239
3	170	79	144	79	0.96	<0.001	−0.01	0.153

[1]Pearson ($P \leq 0.05$).
[2]Analyzed as $\log_{10}$, paired *t*-test ($P \leq 0.05$).

duplicate using the portable electronic counter DCC exhibited correlation of 0.99, showing that without reader influence, results had less variation (Sarikaya and Bruckmaier, 2006).

Results of Somaticell test evaluated by duplicate and reader were combined for analysis. There was no difference in $\log_{10}$ of Somaticell readings by duplicate ($P = 0.500$) or reader ($P = 0.268$), nor was the interaction between duplicate and reader significant ($P = 0.715$). The observed within-reader variation of reader 1 was not able to affect $\log_{10}$ Somaticell results when comparing duplicates and readers. The mean $\log_{10}$ of Somaticell values for duplicate 1 and 2 were 5.01 and 4.99, respectively, and were 5.03, 4.98, and 5.00, for reader 1, 2, and 3, respectively. Using a threshold of 205,000 cells/mL to define subclinical mastitis, results of Somaticell test within reader were classified as having or not having infection (Figure 3). Readers 2 and 3 demonstrated the same proportion of results classified as mastitis and healthy, but reader 1 recorded 7.9% (duplicate 1) and 6.8% (duplicate 2) more infected samples. Analyzing this data stratified by duplicate, there was no significant difference among readers in classifying samples based on duplicate ($P > 0.182$). Although reader 1 had assigned more samples to the mastitis group, this difference did not affect the general classification of samples.

In conclusion, results of Somaticell test were similar to results of electronic counting, and a high degree of agreement was seen when a threshold of 205,000 cells/mL was used to define IMI. Validity of Somaticell test for quarter milk samples was adequate, and the SCC readings were associated with results of microbiological culture. Variation of test results was observed but did not affect the classification of samples for subclinical mastitis. Future work needs to be done to evaluate the use of Somaticell test with both composite and bulk milk samples for determination of equivalent SCC.

On-farm tests for SCC should be chosen according to result applicability for management decisions, taking in account test performance, utility, and cost. Usually tests that are portable, rapid to perform, and inexpensive are preferred, but results should be looked at carefully mainly when using qualitative tests. Results of quantitative tests have greater correlation with electronic SCC, and miniaturized electronic counters are the most recent choice for on-farm SCC determination.

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