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Evaluation and Comparison of 2 On-Farm Tests for Estimating Somatic Cell Count in Quarter Milk Samples from Lactating Dairy Cattle

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Background: The somatic cell count (SCC) is commonly used to monitor udder health and diagnose subclinical intramammary infection (IMI) in dairy cattle.

Hypothesis: The Somaticell test (ST)[b] and California mastitis test (CMT) are clinically useful cow-side tests for diagnosing subclinical IMI.

Animals: One hundred and eleven dairy cows at dry-off and 92 cows within 4–7 days postcalving.

Methods: Quarter foremilk samples were obtained and analyzed with a DeLaval cell counter (DCC, reference method),[a] ST, and CMT. The ST was run in a simulated cow-side manner using milk at 37°C instead of 0–8°C as recommended by the manufacturer. Test performance for diagnosing IMI (DCC SCC >200,000 cells/mL) was evaluated by calculating the area under the receiver operating characteristic curve (AUC) and the kappa coefficient (κ) at the optimal cut-point for each test. The effect of milk/reagent temperature also was evaluated.

Results: Compared to the reference method, the ST run in a simulated cow-side manner had an AUC = 0.68 and κ = 0.24 at dry-off, and AUC = 0.74 and κ = 0.40 in fresh cows. The CMT performed much better than the ST in diagnosing subclinical IMI with AUC = 0.88 and κ = 0.77 at dry-off, and AUC = 0.87 and κ = 0.76 in fresh cows. The measured ST value decreased with increasing temperature of the milk/reagent mixture.

Conclusions/Clinical Importance: The ST is optimized for use on milk at 0–8°C and is therefore designed for on-farm use on refrigerated milk samples. The ST is not suited for use as a cow-side screening test for IMI because the milk temperature exceeds the recommended range for the test.

Key words: California mastitis test; DeLaval cell counter; Intramammary infection; Somaticell test.

The somatic cell count (SCC) is the most commonly used method for evaluating milk quality and overall udder health in dairy cattle[1,2] because of the association between the number of inflammatory cells in milk and the presence of intramammary infection (IMI). The SCC is defined as the concentration of leukocytes and epithelial cells in milk and is expressed as "cells per mL of milk."[3] Leukocytes are present to facilitate the removal of invading pathogens, and epithelial cells are continuously shed from glandular tissue into milk. As a consequence, healthy quarters without IMI have a SCC ranging from 10,000 to 100,000 cells/mL.[4] In the presence of IMI, leukocytes are recruited to move from the circulation into milk, resulting in an increased SCC. Monitoring the SCC therefore has been used for decades to identify the presence of IMI and assess milk quality.

Abbreviations:

AUC	area under the curve
CMT	California mastitis test
DCC	Delaval cell counter
IMI	intramammary infection
ROC	receiver operating characteristic curve
SCC	somatic cell count
Se	sensitivity
Sp	specificity
ST	Somaticell test
UIDRF	University of Illinois Dairy Research Farm
WMT	Wisconsin mastitis test

On-farm SCC tests are useful screening methods that permit dairy producers to improve udder health and milk quality by making management decisions in a timely manner. The DeLaval Cell Counter (DCC)[a] is an accurate portable optical cell counter that is designed to be used on-farm for rapid SCC evaluation, providing a result in 45 seconds.[5] The DCC has been validated as being equivalent to the Fossomatic and direct microscopic methods when analyzing bovine milk samples at 4°C.[6,7] The CMT is a cow-side, semiquantitative screening test that has been used for 60 years and provides a result within 1 minute, although there is high variability in SCC within each score. The Somaticell test (ST)[b] is a modified version of the Wisconsin mastitis test (WMT)

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that can be performed on-farm to quantify the SCC within 2 minutes.[8–10] The test was made available in the United States (US), Canada, and Latin America in February 2015. The manufacturer recommends that milk samples be 0–8°C when tested by the ST, which requires refrigeration after sample collection. Because an accurate cow-side test would be very helpful in directing the need for intramammary antibiotic infusion at dry-off or at freshening, we were interested in evaluating the clinical utility of the ST on milk samples at approximately 37°C. We hypothesized that the ST, when used other than as intended by the manufacturer, would provide a clinically useful quantitative cow-side test for SCC and therefore be helpful in predicting the presence of IMI in dairy cows at dry-off and during the first week of lactation.

We were interested in addressing this hypothesis for 3 reasons. First, identification of a clinically useful test would assist the dairy industry in the goal of decreasing the amount of intramammary antibiotics administered to food-producing animals. The use of dry cow treatment (DCT) for every quarter of every cow (blanket DCT) is a cornerstone of the mastitis control program and management strategy in North America.[11] This practice is effective in decreasing the prevalence of IMI within a herd by eliminating existing IMIs and preventing new infections during the dry period.[12] Although not documented to be as a consequence of DCT, the potential emergence of antibiotic-resistant strains of bacteria is one of the major arguments against the use of blanket DCT.[12,13] This concern, coupled with a societal desire to decrease antibiotic administration to production animals, has led to increased interest in selective DCT, particularly in conjunction with the use of internal teat sealants to prevent new IMI during the dry period.[14,15] Therefore, the accurate identification and treatment of infected cows at dry-off or in early lactation remain an important goal of mastitis control programs. Unfortunately, accurate, practical, objective, and low-cost methods to determine the udder health status of cows at dry-off and in early lactation have yet to be identified. Second, the World Health Organization has published guidelines for the development of diagnostic tests for infectious agents in resource-poor settings, such as dairy farms. The diagnostic tests must be affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and delivered to those in need, providing the acronym "ASSURED."[16] Point-of-care diagnostic tests in resource-limited settings therefore should be sufficiently accurate, have immediate clinical impact, and be cost-effective.[17] On this basis, the ST should be compared to an on-farm quantitative SCC instrument, such as the DCC, as well as a cow-side semiquantitative test, such as the CMT. Third, although the WMT provides a more accurate estimate of milk leukocyte count than does the CMT when performed in a laboratory,[18] the effect of temperature on the WMT has not been well documented. The available information suggests that the WMT provides optimal results when the final solution temperature is 24 ± 2°C[19,20] and that any effect of temperature on the WMT should be quantifiable. In other words, even if temperature impacted the ST reading when used in a cow-side setting, it was likely that the measured value could be corrected for a temperature effect and thereby provide clinically useful information. Our primary objective was therefore to evaluate the clinical performance of the ST as a cow-side semiquantitative screening test for estimating SCC in dairy cows at dry-off and during the first week of lactation. These 2 time periods were selected for investigation because decisions are made at these time points as to whether intramammary antibiotics should be infused to treat an IMI or not. Secondary objectives were to investigate the effect of milk sample and reagent temperature on the ST and to compare the clinical performance of the ST used in a simulated cow-side manner against the CMT.

Materials and Methods

Animals, Housing, and Milking System

An observational study was conducted on a convenience sample of 124 lactating cattle, 111 of which were sampled during the last week of lactation, and 98 were fresh cows that were sampled 4–7 days postpartum. The study was performed at the University of Illinois Dairy Research Farm (UIDRF) from July 1, 2015, to July 31, 2016. The average herd size during the study period was 136 dairy cows. Late gestation cows were housed outdoors in a dry lot and were moved indoors to a calving pen when parturition was imminent. After calving, all cows were kept in a tie-stall barn for at least 3 days before being moved to a free stall with the lactating herd. Cows were fed a dry cow or a lactating cow total mixed ration based on formulations recommended by the National Research Council[21], and milked 3 times daily in a milking parlor at 05:00, 14:00, and 21:30. Before the cow was milked, each teat was dipped into a premilking teat dip containing lactic acid[e] and dried using single-service towels. After milking, each teat was dipped into a postmilking teat dip containing an iodine-based product[d] and allowed to air-dry. Cows with abnormal milk or udder were identified as clinical mastitis cases by the milkers and not sampled or included in the study. The average monthly incidence of clinical mastitis was 4.7% and the average bulk milk SCC during the period of study was 249,000 cells/mL. A dry cow intramammary ceftiofur formulation[e] and a dry cow teat sealant[f] were applied to all cows at dry-off. Cows also were vaccinated with an Rc core-lipopolysaccharide antigen vaccine[g] at dry-off. All methods were evaluated and approved by the University of Illinois Institutional Animal Care and Use Committee.

Experimental Methods

The dairy was visited once per week to collect foremilk samples from all quarters of selected cows. A clinical examination was performed on each cow and udder before obtaining milk samples, and abnormalities were recorded. The milk samples were collected from late lactating cows once on the same week before being dried off between 12:00 and 16:00, and once after calving (from day 4 to 7 postcalving) between 12:00 and 14:00. Milk samples were collected from each quarter aseptically after cleaning the teat end with a sterile gauze swab and 70% alcohol.[22] Samples were collected from all 4 quarters within 50 seconds of first touching a teat to ensure that samples reflected cistern milk and not a mixture of cistern and alveolar milk due to endogenous oxytocin release.[23] Quarter samples were collected into sterile labeled tubes by hand

stripping after discarding the first 3 squirts of milk. Samples then were stored in an insulated box containing ice water for transportation to a laboratory at the UIDRF and then to a second laboratory at the College of Veterinary Medicine.

Somatic Cell Count Determination

The reference method for determining SCC was the DCC.[a] The DCC is a portable cell counter that counts somatic cells optically, with a reported measurement range of 10,000 to 4,000,000 cells/mL. The manufacturer reported a coefficient of variation of 12% at 100,000 cells/mL, 8% at 400,000 cells/mL, and 7% at 1,000,000 cells/mL. In a separate study, the reference method was linear with the Fossomatic and direct microscopic tests when analyzing bovine milk samples at 0–6°C, but read approximately 12% lower than those 2 tests.[24] Samples were measured with the DCC at the UIDRF within 2 hours of collection at room temperature (approximately 20°C; estimated range, 15–30°C) as recommended by the manufacturer. Milk was drawn up using a piston into a single-use cassette that then was inserted into the DCC unit and analyzed. The reported SCC value in cells/μL of milk was multiplied by 1,000 to provide SCC in units of cells/mL of milk.

The ST[a] was used to measure SCC according to instructions of the manufacturer except that the milk sample temperature was approximately 37°C instead of 0–8°C as recommended by the manufacturer. Because of workload constraints during the course of the study, the SCC was measured on quarter milk samples within 4 hours of collection after transport back to the laboratory at the College of Veterinary Medicine. The ST is a modified version of the WMT that provides a semiquantitative estimate of SCC using a calibrated scale for SCC based on 41 outcomes for SCC ranging from 69,000 cells/mL to 1,970,000 cells/mL.[25] The test materials included single-use polypropylene calibrated analysis tubes, caps with drainage holes, mixing straws, and reagent. Quarter milk samples were placed in a water bath at 37°C for at least 30 minutes to simulate milk temperature when used as a cow-side test. Two mL of the test reagent (at room temperature of approximately 20°C) was added to the calibrated tube followed by addition of 2 mL of a well-mixed quarter milk sample at 37°C. The milk and reagent in the tube then were mixed by moving the straw 20 times up and down in 30 seconds. The tube was closed with the perforated cap and inverted for 30 seconds to permit drainage of the non-coagulated solution, returned to a vertical upright position, and allowed to stand for a few seconds for liquid to settle before reading. The level of milk remaining in the tube was read, with the scale number on the tube at the milk level indicating the estimated SCC in thousands/mL. The time difference of up to 4 hours between sample collection and analysis was expected to minimally impact the measured SCC because the manufacturer recommends using the ST on milk stored for up to 36 hours. Moreover, because the ST is a modified version of the WMT, and the SCC measured by the WMT decreases by 5–10% during storage at 0–4°C for 24 hours,[19] the estimated maximal decrease in SCC during the mean 2 hours time difference between DCC and ST measurements was 0.4–0.8%.

The CMT was performed cow-side as described elsewhere.[26] Two mL of fresh foremilk sample from each quarter was placed in the appropriate chamber of the CMT plastic paddle and mixed with 2 mL of CMT reagent at ambient temperature by gently moving the paddle in a circular motion for 30–45 seconds. A change in viscosity indicated an increase in quarter SCC, with the CMT reaction being visually scored by 1 investigator (SK) using a 5-point scale as: negative, trace, 1 positive (1), 2 positive (2), and 3 positive (3).

Effect of Sample Temperature

The ST is a modification of the WMT with some important differences: the tube drainage hole diameter is approximately 0.8 mm for the ST and 1.2 mm for the WMT, the tube is partly conical for the ST but cylindrical for the WMT, and the inversion time is 30 seconds for the ST and 15 seconds for the WMT.[19,20,27] The recommended temperature for the quarter sample is 0–8°C for the ST (manufacturer's recommendation) and 0–4°C for the WMT.[19] The ST manufacturer, however, recommends use of reagent solution at room temperature (18–26°C) with a resultant final solution temperature of approximately 14°C. For comparison, the WMT recommends use of reagent solution at 45°C so that the resultant final solution temperature is 24 ± 2°C.[19,20]

The effect of final solution temperature on the ST was investigated using 10 randomly collected 20 mL composite milk samples obtained from Holstein-Friesian cows during milking in the parlor. The temperature of the 2 mL composite milk samples was equilibrated to approximately 3, 20, and 37°C by placing the sample in the refrigerator, at room temperature, or in a water bath at 37°C, respectively, for 30 minutes. The reagent solution was equilibrated to approximately 20 and 45°C by placing the solution at room temperature or in a water bath at 45°C, respectively, for 30 minutes. The ST then was run as previously described on the following milk/reagent temperature combinations for 4 aliquots from each cow, in ascending order of final mixture temperature: 3/20°C; 20/20°C, 3/45°C, 37/20°C.

Statistical Analysis

Data were expressed as median and range and $P < 0.05$ was considered significant. Statistical analyses were performed by MedCalc Statistical Software version 15.11.4[h] and SAS 9.4[i] . The presence of IMI was defined as SCC >200,000 cells/mL because this is the most frequently used method and cut-off value for diagnosing IMI[28–31] with maximum sensitivity and specificity[32] and minimal diagnostic error.[1] Measured DCC and ST values exceeding the upper value for the measurement interval were assigned that value (ie, samples were not diluted and reanalyzed). Measured ST values below the lower value for the measurement interval (69,000 cells/mL) were assigned that value and depicted graphically but were not included in the Passing-Bablok regression procedure or Bland-Altman plot analysis.

Passing-Bablok regression[33] was used to evaluate the linear relationship between the $\log_{10}$(SCC) measured by the ST and reference method. For Passing-Bablok regression, the intercept value reflects constant bias and the slope reflects proportional bias. Agreement also was examined by Bland-Altman difference plots[34] using the percentage difference in the $\log_{10}$(SCC) relative to the geometric mean of the 2 measurements. The upper and lower limits of agreement were calculated from the bias ± 1.96 × SD. The bias estimate from Bland-Altman plots reflects the mean bias over the range of measured values and therefore includes both the constant and proportional bias identified by Passing-Bablok regression. Based on the imperfect measurement accuracy of the reference methodology and resolution of the ST, we assigned a range of 25% as a priori acceptable limits of agreement for $\log_{10}$(SCC).

Binary logistic regression[35,36] (PROC LOGISTIC, SAS 9.4) was used to characterize the relationship between IMI as determined by the reference method (1 = IMI present, 0 = IMI absent) and SCC measured by the ST, or CMT score, at dry-off and for fresh cows. The adequacy of the logistic regression model fit was evaluated by the Hosmer-Lemeshow goodness-of-fit statistic and plots of deviance influence statistics against the predicted values. Receiver operating characteristic (ROC) curves were constructed for each logistic regression model. The area under the ROC curve

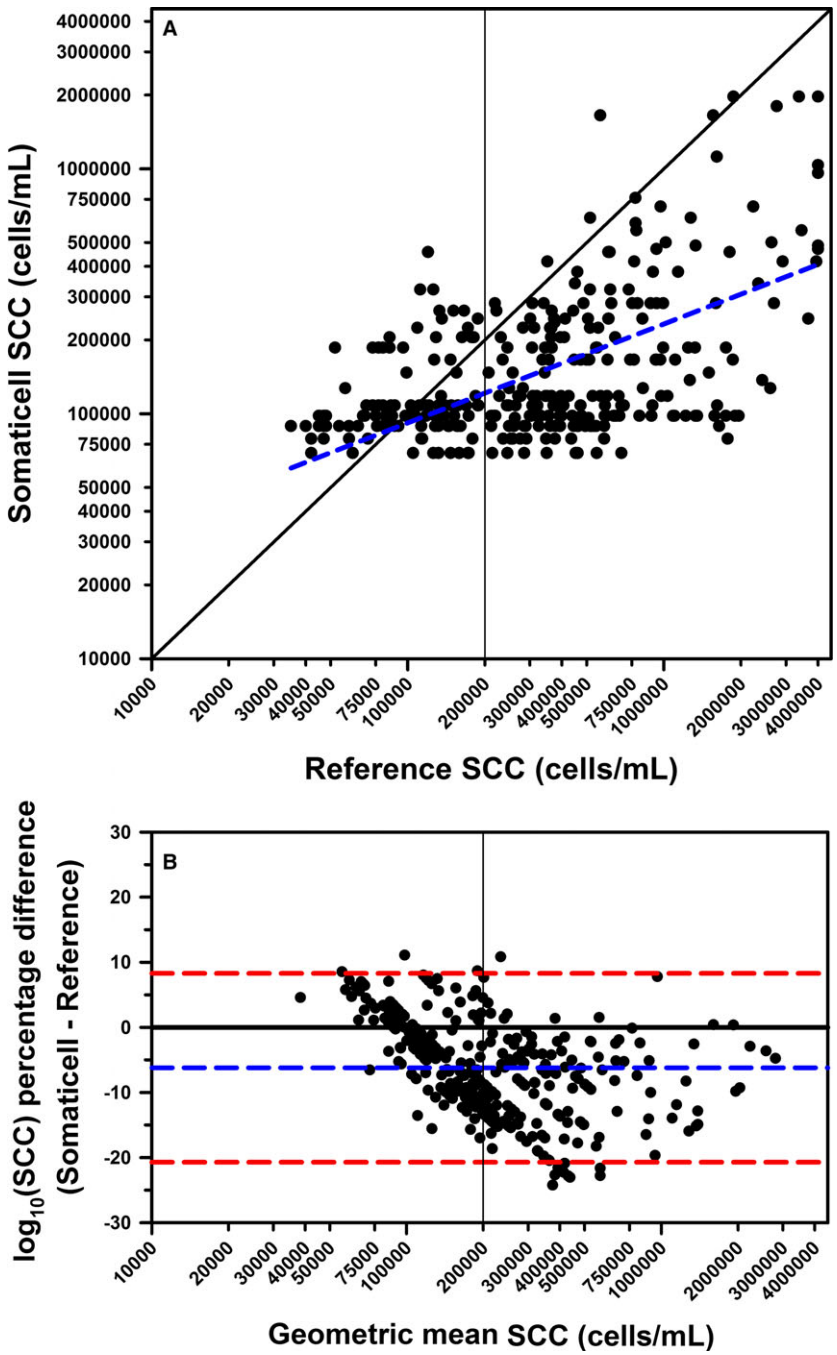

Fig 1. (**A**) Scatterplot of the relationship between the somatic cell count (SCC) measured by the Somaticell test (ST) and reference method for 323 quarter milk samples from 81 dairy cows at dry-off. The diagonal solid black line is the line of identity, and the dashed blue line is the line of best fit from Passing and Bablok regression. (**B**) Bland-Altman plot of the percentage difference between $\log_{10}$ SCC measured by the ST and the reference test against the geometric mean value for SCC. The horizontal dashed blue line is the mean bias (−6.2%), and the horizontal dashed black lines reflect the 95% limits of agreement (−20.7–8.3%), which is equivalent to the range of differences containing 95% of future measurements. The vertical black lines indicate the SCC cut-point for IMI (200,000 cells/mL).

(AUC) was calculated as a global index of test performance. The AUC values for ROC curves >0.9 typically indicate a highly accurate test, whereas AUC values of 0.7–0.9 indicate moderate accuracy, 0.5–0.7 low accuracy, and 0.5 a chance result.[37] Sensitivity (Se) and specificity (Sp) were calculated at the optimal cut-point of the ROC determined by the Youden index (the cut-point where the following expression has its maximum value: Se + Sp − 1), which equally weights Se and Sp. The Kappa coefficient (κ, PROC FREQ, SAS 9.4) then was calculated at the optimal cut-point to further characterize the level of agreement between the tests. Values for $\kappa \leq 0.2$ indicate poor agreement, whereas $0.2 < \kappa \leq 0.4$ indicates fair agreement, $0.4 < \kappa \leq 0.6$ indicates moderate agreement, $0.6 < \kappa \leq 0.8$ reflects good agreement, and $\kappa > 0.8$ indicates excellent agreement.[38]

The effect of milk sample and reagent solution temperature on the ST result was investigated by paired *t*-tests, with the last 3

combinations compared separately to the manufacturer's recommended temperature combination (milk at 3°C, reagent solution at 20°C).

Results

Quarter milk samples were obtained at dry-off from 111 cattle, comprising 99 Holstein-Friesian, 8 Jersey, 2 Ayrshire, 1 Brown Swiss, and 1 Milking Shorthorn. The median SCC value at dry-off measured by the reference method was 364,000 cells/mL for 443 quarter samples, with 1 cow having a blind quarter (no sample available). The prevalence of IMI, defined as SCC >200,000 cells/mL on a quarter basis at dry-off, was 69% (304/443).

Quarter milk samples were obtained at freshening from 92 cattle, comprising 81 Holstein-Friesian, 8 Jersey, 1 Ayrshire, 1 Brown Swiss, and 1 Milking Shorthorn. The median SCC value at freshening measured by the reference method was 113,000 cells/mL for 364 quarter samples, with 4 cows having 1 blind quarter. The prevalence of IMI defined as SCC >200,000 cells/mL on a quarter basis at freshening was 33% (120/364).

Somaticell Test

Quarter milk samples were obtained at dry-off from 81 of the 111 cattle, comprising 72 Holstein-Friesian, 6 Jersey, 1 Ayrshire, 1 Brown Swiss, and 1 Milking Shorthorn. Samples were not obtained from 20 cattle because of delays in obtaining the ST. The median SCC measured by the ST at dry-off (323 quarter samples, with 1 cow having a blind quarter) was 108,000 cells/mL. The prevalence of IMI in quarters submitted to the ST was 66% (213/323).

Passing and Bablok regression of the comparison between measured $\log_{10}$(SCC) by the ST and reference method at dry-off indicated a proportional bias of 0.40 (95% confidence interval [CI]: 0.33–0.48) that was <1 and a constant bias of 2.94 (equivalent to a SCC of 871 cells/mL; 95% CI: 2.51–3.34) that was >0 (Fig. 1A). The accompanying Bland-Altman plot indicated that the ST value for $\log_{10}$(SCC) was 6.2% lower than the reference method with a mean bias of −6.2% ($P < 0.0001$ compared with 0) and 95% limits of agreement from −20.7 to 8.3% (Fig. 1B). The range for the 95% limits of agreement was within the 25%, regarded a priori as being acceptable. Logistic regression analysis on the 323 quarter samples obtained at dry-off indicated that 123,864 cells/mL provided the optimal cut-point for using the ST to identify an IMI based on reference SCC >200,000 cells/mL, equivalent to an ST reading >118,000 cells/mL. Using this cut-point, AUC = 0.68, Se = 0.60, Sp = 0.74, and $\kappa = 0.24$ (Fig. 2, left panel).

Quarter milk samples were obtained at freshening from 60 of the 92 cattle, comprising 53 Holstein-Friesian, 6 Jersey, and 1 Ayrshire. Samples were not obtained from 32 cattle at freshening because of delays in obtaining the ST. The median SCC measured by the ST at freshening (237 quarter samples) was 108,000 cells/mL. The prevalence of IMI in quarters submitted to the ST was 32% (77/237) at freshening.

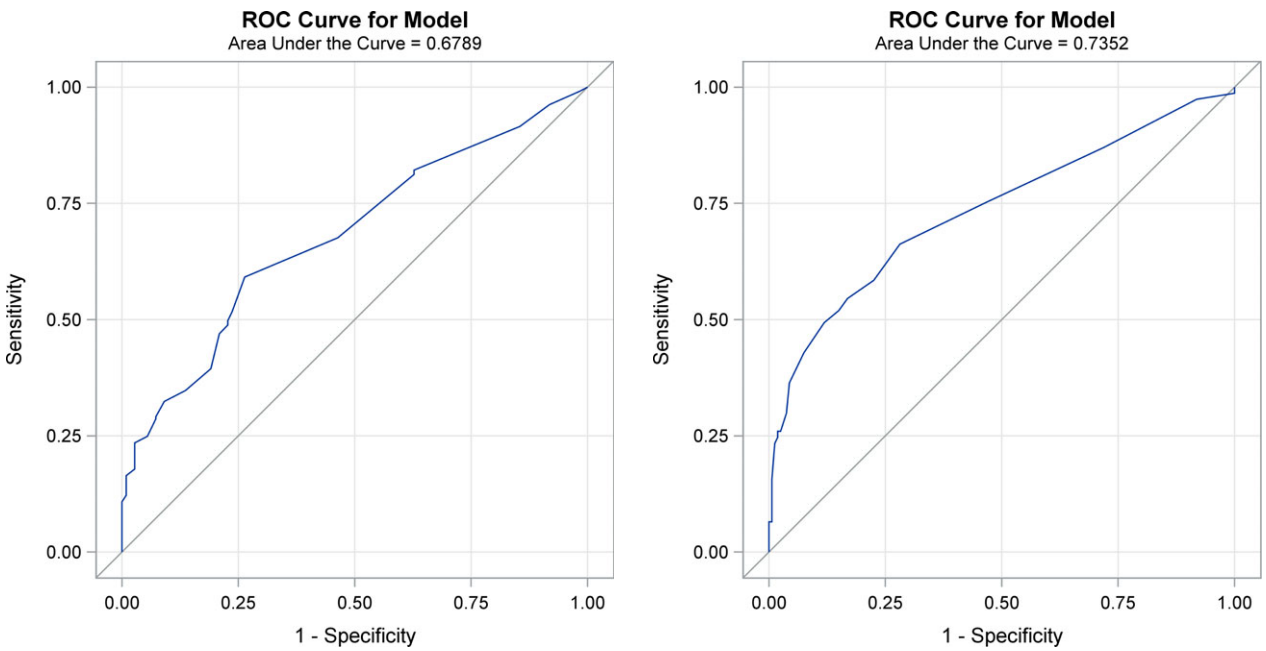

Fig 2. Left panel—Receiver operating characteristic (ROC) curve for the Somaticell test (ST) in detecting intramammary infection (IMI) in 323 quarters from 81 cows at dry-off. The optimal cut-point for detecting an IMI was a ST result of >118,000 cells/mL (area under the ROC curve = 0.68; sensitivity = 0.60; specificity = 0.74). Right panel - Receiver operating characteristic curve for the ST in detecting IMI in 237 quarters from 60 cows at freshening. The optimal cut-point for detecting an IMI was a ST result of >166,000 cells/mL (area under the ROC curve = 0.74; sensitivity = 0.66; specificity = 0.72).

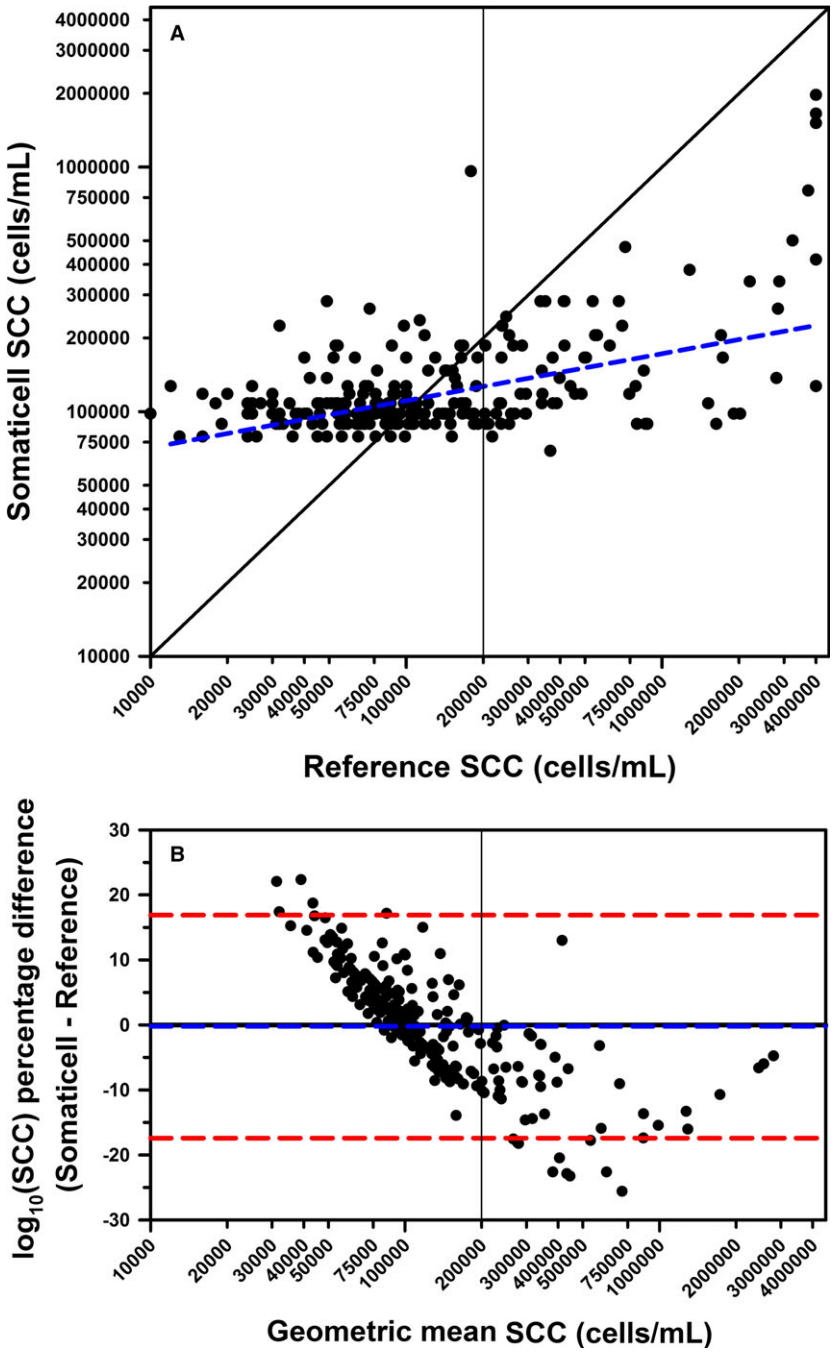

Fig 3. (**A**) Scatterplot of the relationship between Somaticell test (ST) somatic cell count (SCC) and the SCC determined by the reference method for 237 quarter milk samples from 60 fresh dairy cattle. The solid diagonal line is the line of identity, and the dashed line is the line of best fit from Deming regression. (**B**) Bland-Altman plot of the percentage difference between $\log_{10}$ SCC measured by the ST and reference method against the geometric mean value for SCC. The horizontal short dashed line is the mean bias (−0.2%), and the horizontal long dashed lines reflect the 95% limits of agreement (−17.4–16.9%), which is equivalent to the range of differences containing 95% of future measurements. The vertical black lines indicate the SCC cut-point for IMI (200,000 cells/mL).

Passing and Bablok regression of the comparison between measured $\log_{10}$(SCC) by the ST and reference method in 237 quarter milk samples obtained from day 4 to 7 of lactation indicated a proportional bias of 0.19 (95% CI: 0.14–0.25) that was <1 and a constant bias of 4.08 (equivalent to a SCC of 12,023 cells/mL; 95% CI: 3.81–4.33) that was >0 (Fig. 3A). The accompanying Bland-Altman plot indicated that the ST value for $\log_{10}$(SCC) was similar to the reference method with a mean bias of −0.2% ($P = 0.24$ compared with 0) and 95% limits of agreement from −17.4 to 16.9% (Fig. 3B). The range for the 95% limits of agreement (34.3%) exceeded the 25% regarded a priori as being acceptable. Logistic regression analysis on the samples

obtained from fresh cows indicated that 173,800 cells/mL provided the optimal cut-point for using the ST to identify an IMI based on reference SCC >200,000 cells/mL, equivalent to an ST reading >166,000 cells/mL. Using this cut-point, AUC = 0.74, Se = 0.66, Sp = 0.72, and $\kappa = 0.40$ (Fig. 2, right panel).

California Mastitis Test

At dry-off, 28.7, 25.3, 23.5, 5.8, and 6.8% of quarters had CMT scores of 0, 0.5, 1, 2, or 3, respectively, with median SCC of 107,000, 313,500, 538,000, 1,278,500, and 2,105,000 cells/mL, respectively, as measured by the reference method (Fig. 4, top panel). Logistic regression analysis on the 443 quarter samples obtained at dry-off indicated that a CMT score ≥trace provided the optimal cut-point for using the CMT to identify an IMI based on reference SCC >200,000 cells/mL. Using this cut-point, AUC = 0.88, Se = 0.95, Sp = 0.81, and $\kappa = 0.77$ (Fig. 5, left panel).

In fresh cows, 70.3, 15.9, 6.6, 3.6, and 3.6% of quarters had CMT scores of 0, 0.5, 1, 2, and 3, respectively, with median SCC of 79,000, 330,500, 730,500, 1,731,000, and 4,000,000 cells/mL, respectively, as measured by the reference method (Fig. 4, bottom panel). Logistic regression analysis on the 364 quarter samples obtained at freshening indicated that a CMT score ≥trace provided the optimal cut-point for using the CMT to identify an IMI based on reference SCC >200,000 cells/mL. Using this cut-point, AUC = 0.87, Se = 0.79, Sp = 0.95, and $\kappa = 0.76$ (Fig. 5, right panel).

Effect of Sample Temperature

Measured temperatures for the 4 milk-reagent combinations were 15.2 ± 0.6°C for 3/20°C; 20.1 ± 0.3°C for 20/20°C; 22.2 ± 0.6°C for 3/45°C; and 25.9 ± 0.6°C for 37/20°C (Fig. 6).

The geometric mean SCC measured by the ST at the recommended milk-reagent mixture temperature (3/20°C) was similar to that measured by the DCC. The temperature of the milk-reagent mixture when analyzed impacted the SCC value provided by the ST (Fig. 6), with the measured value for SCC being decreased at mean milk-reagent temperatures of 22.2°C and 25.9°C, obtained by milk/reagent temperature mixtures of 3/45°C and 37/20°C, respectively.

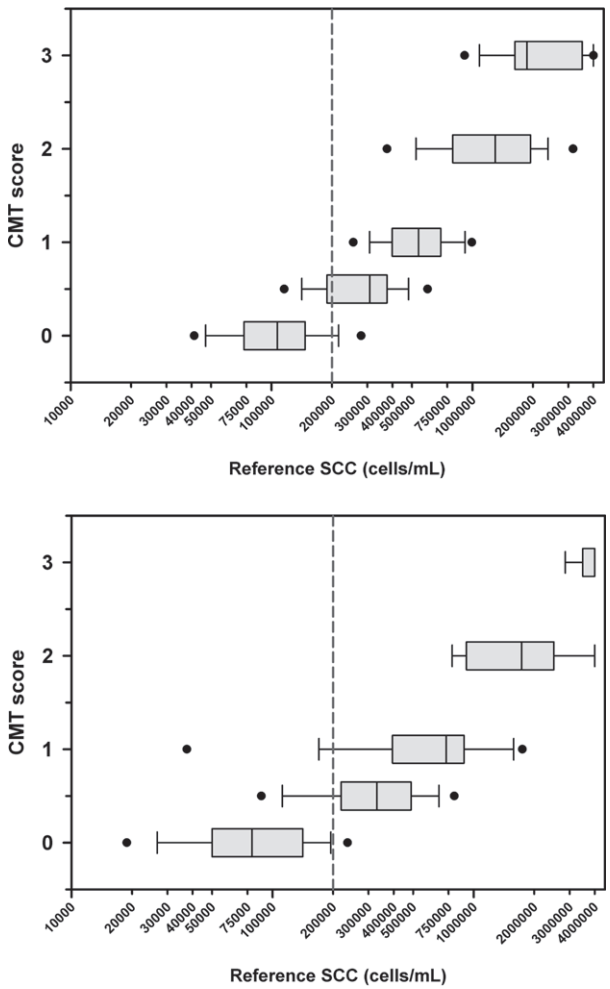

Fig 4. Top panel—Box and whiskers plot of the association between the California mastitis test (CMT) value and somatic cell count (SCC) measured by the reference method in 443 quarters from 111 dairy cattle at dry-off. The shaded box represents the first and third quartile, the vertical line in the shaded box represents the median value, the whiskers represent the 10th and 90th percentiles, and filled circles represent data points outside this percentile range. The vertical dashed black line indicates the SCC cut-point for intramammary infection (200,000 cells/mL). The CMT score was categorized as 0, Trace (T), 1, 2, or 3.Bottom panel—Box and whiskers plot of the association between the CMT value and SCC measured by the reference method in 364 quarters from 92 cattle at freshening.

Discussion

Our study compares the clinical utility of the ST when used other than as intended and the CMT as cow-side tests for diagnosing IMI defined as SCC >200,000/mL in dairy cows at dry-off and at freshening. Our first major finding was that the ST when run in a simulated cow-side manner contrary to manufacturer's instructions markedly underestimated the SCC, particularly when SCC exceeded 200,000 cells/mL. The second major finding was that the CMT, when used at a cut-point of trace or higher, had a much higher test sensitivity and specificity than the ST used in a simulated cow-side manner at dry-off and at freshening. The CMT therefore provides a faster and more accurate cow-side screening test to predict IMI defined as SCC >200,000 cells/mL at dry-off and freshening than does the ST used in a simulated cow-side manner.

The specific procedure of test mixing that involves a combination of equal 2 mL volumes at different temperatures is suspected to be a major point of test variability that affects the performance of the ST.[9] Although not well documented, the original description of the WMT recommended that the temperature of the milk-reagent mixture be 24 ± 2°C,[19,20,27] which reflected the

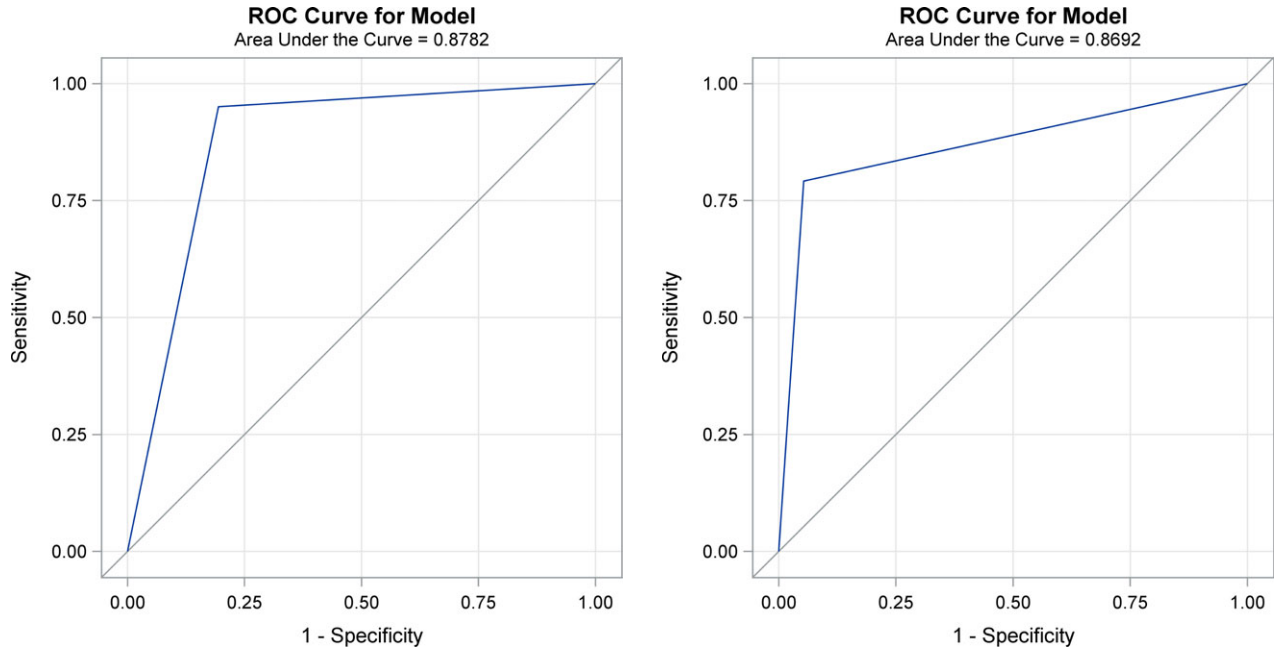

Fig 5. Left panel—Receiver operating characteristic (ROC) curve for the California mastitis test (CMT) in detecting intramammary infection (IMI) in 443 quarters from 111 cows at dry-off. The optimal cut-point for detecting an IMI was CMT ≥trace (area under the ROC curve = 0.88; sensitivity = 0.95; specificity = 0.81). Right panel—Receiver operating characteristic curve for the CMT in detecting IMI in 364 quarters from 92 cows at freshening. The optimal cut-point for detecting an IMI was CMT ≥trace (area under the ROC curve = 0.87; sensitivity = 0.79; specificity = 0.95).

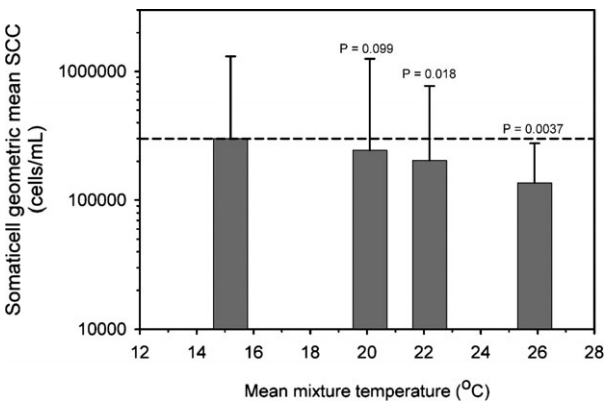

Fig 6. Bar graph of the geometric mean somatic cell count (SCC) for the Somaticell test (ST) run at 4 milk/reagent temperature combinations (3/20°C; 20/20°C, 3/45°C, 37/20°C) on composite milk samples from 10 Holstein-Friesian cows. The dashed horizontal line is the geometric mean SCC for the 10 composite milk samples measured by the Direct Cell Counter (DeLaval, Tumba, Sweden). The error bars represent the upper bound of the 95% confidence interval for the sample population. The *P* values represent the difference in geometric mean SCC at a specific milk/reagent combination compared to the temperature recommended by the manufacturer of the ST (milk, 3°C; reagent, 20°C).

use of the ST in our study because mixing milk samples at 37°C with reagent at room temperature produced a final mean milk-reagent temperature of 25.9°C. The relatively poor performance of the ST in our study may have been due to the quarter sample being at approximately 37°C when tested to mimic use as a cow-side test, instead of 0–8°C as recommended by the test manufacturer, because we determined that higher milk-reagent temperatures resulted in lower SCC values by the ST. Higher sample temperatures would be expected to decrease viscosity and increase the amount of noncoagulated fluid draining from the tube during inversion for 30 seconds, leading to less fluid retained in the tube and an artificially lower SCC. Alternatively, the unanticipated poor performance of the ST may have been due to the presence of many quarter samples at dry-off having SCC ranging from 214,000 to 647,000 cells/mL, because the WMT has decreased accuracy in this SCC range.[20] Other studies have demonstrated that the ST run on milk samples at 0–8°C performs reasonably well when SCC < 200,000 cells/mL, but under reports the SCC when SCC >200,000 cells/mL.[9,10] Interestingly, the ST performed better in our study with milk samples obtained at freshening, possibly because there were relatively fewer milk samples with SCC ranging from 214,000 to 647,000 cells/mL or the sample had a different viscosity than that at dry-off. The results of another study indicated that the ST provided a useful measure of SCC in bulk tank milk that was refrigerated and analyzed within 24 hours of collection.[39] Whatever the reason for the suboptimal performance of the ST when used contrary to instructions in our study, the logistic regression procedure adjusts the optimal cut-point for the test in diagnosing IMI and at the optimal cut-point (>118,000 cells/mL at dry-off and >166,000 cells/mL at freshening), the calculated sensitivity and specificity

values were likely to be similar to those obtained at 0–8°C, unless analyzing quarter samples at 37°C markedly increased the sample-to-sample variability in viscosity.

The unit of analysis in our study was the quarter. Comparison of test performance was based on the assumption that sensitivity and specificity were of equal importance, and on this basis, the AUC and κ coefficient provide useful clinical indices of overall test performance. The AUC for the ST indicated low-to-moderate accuracy (0.68 at dry-off and 0.74 at freshening). In contrast, the CMT was a moderately accurate test at a trace reaction or higher (AUC, 0.88 at dry-off; AUC, 0.87 at freshening). Similarly, the κ coefficient indicated fair agreement between the ST results and the reference method (0.24 and 0.40 in dry-off and fresh cows, respectively) in classifying quarter samples by infection status. For comparison, the κ coefficient indicated good agreement between the CMT and reference method (0.77 at dry-off and 0.76 at freshening) in classifying quarter samples by infection status.

In our study, the CMT showed good sensitivity (95 and 79% at dry-off and in fresh cows, respectively) and specificity (81 and 95% at dry-off and in fresh cows, respectively) using a threshold reaction >0 (ie, any non-negative CMT score). The clinical utility of using the CMT to diagnose subclinical IMI therefore is optimized by interpreting the test as negative or positive (trace, score 1, score 2, and score 3) to achieve the highest sensitivity with acceptable specificity. The CMT was read by 1 investigator (SK) for the entire study, and the subjective nature of interpreting the CMT may result in different sensitivity and specificity estimates by other users. However, our results were similar to those reported for 3,012 quarter milk samples from 760 lactating cows in Brazil, where average SCC in cells/mL for CMT scores were as follows: 79,900 for CMT = 0; 333,500 for CMT = trace; 670,300 for CMT = 1; 1,354,000 for CMT = 2; and 4,455,600 for CMT = 3.[40] In addition, using a reference SCC of 200,000 cells/mL as an indication of IMI, the sensitivity was 79% and specificity was 90% in the other study.[40] Our results also were consistent with the following median SCC in cells/mL for CMT scores in Brown Swiss cows in Turkey: 21,500 for CMT = 0; 340,500 for CMT = 1; 1,069,000 for CMT = 2; and 3,948,500 for CMT = 3.[41]

We are not aware of a study that identifies an effect of breed on the ST or CMT, separate from any potential breed effect on SCC. The proportion of cattle in various dairy breeds in our study approximates that of the US dairy industry, and consequently, our results should be generalizable to dairy cattle in the United States. An effect of breed on the test performance of ST or CMT is considered unlikely because viscosity in both tests is driven primarily by the interaction of DNA derived from somatic cells.[26] Maximum gel formation in the CMT occurs at 60–150 seconds. This response is attributed to the time required for the anionic surfactant to break the cells open, release the DNA, and for anionic surfactant-DNA binding to occur through pH-dependent ionic interactions.[42,43] The WMT uses an anionic surfactant similar to that of the CMT, and consequently, viscosity in the ST is likely to be primarily determined by SCC rather than breed differences in milk fat or protein percentage.

The current costs of the 3 SCC tests used in our study are $0.04, $1.35, and $2.33 for the CMT, ST, and DCC test, respectively, although the DCC test cost does not include the purchase cost of the analyzer. Because of its cow-side application, much lower cost, and acceptable sensitivity and specificity values, the CMT has many of the desirable features of a point-of-care diagnostic test in resource-poor settings, in that it is affordable, sensitive, specific, user-friendly, rapid, and robust,[16] while being sufficiently accurate, cost-effective, and providing immediate clinical impact.[17]

We conclude that the ST is optimized for use on milk at 0–8°C and is therefore not suitable for use as a cow-side screening test to predict IMI at dry-off and freshening where the milk temperature approximates 37°C when tested. In contrast, the CMT provides a clinically useful low-cost cow-side method for diagnosing subclinical IMI in dairy cows at dry-off and early lactation.

Footnotes

[a] DCC, DeLaval, Tumba, Sweden
[b] Somaticell SCC Test, Idexx Laboratories, Inc., Westbrook, Maine, USA
[c] Wash & Prep RTU; Ecolab Inc., MN
[d] Legend; Ecolab Inc., MN
[e] Spectramast DC; Zoetis Animal Health, NJ
[f] Orbeseal; Zoetis Animal Health, NJ
[g] Enviracor J-5 vaccine; Zoetis Animal Health, NJ
[h] MedCalc Software bvba, Ostend, Belgium, 2015
[i] SAS 9.4 software; SAS Inc, Cary, NC

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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